TESTING OF SEED PRE-GERMINATION TREATMENTS FOR SELECTED NATIVE SHRUB SPECIES

Preliminary Phase

by

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ABSTRACT

The results of preliminary experiments on pre-germination treatment methods for nine selected native woody plant species are presented. The species investigated include Amelanchier alnifolia (saskatoon), Arctostaphylos uva-ursi (bearberry), Elaeagnus commutata (silverberry), Juniperus communis (common juniper), Rosa acicularis (prickly rose), Rosa woodsii (Fendler woods rose), Rubus parviflorus (thimbleberry), Rubus strigosus (wild red raspberry) and Shepherdia canadensis (russet buffalo berry).

These studies represent a portion of a program to evaluate, select and test native trees and shrubs for disturbed land reclamation in the Rocky Mountain Eastern Slopes of Alberta.

ACKNOWLEDGMENTS

The authors acknowledge the following people for the invaluable assistance they provided to the project: Mrs. Deborah Smith for her diligent work in preparing seed samples; Mr. Ben Wang (Petawawa Forest Experiment Station), Dr. Paul Ziemkiewicz (Reclamation Research Technical Advisory Committee) and Dr. Al Fedkenheuer (Canstar Oilsands Ltd.) for technical advice and criticism; Mr. Art Evans, Mr. Fred Sutherland, Mr. Jim Molnar and Mr. Ted Loblaw (Alberta Forest Service) for their assistance in locating and collecting seed lots; and the staff of the Alberta Energy and Natural Resources Library for acquisition of published material.

1. GENERAL INTRODUCTION

In the foothills and Rocky Mountain Eastern Slopes of Alberta, renewable resources such as water, recreation, wildlife production and timber production are of critical importance. However, they must be managed simultaneously with non-renewable land uses such as coal mining, oil and gas activities, gravel extraction and other industrial land disturbances. Because of the continuing yield from renewable resources, Alberta non-renewable resource development policy requires restoration to pre-disturbance status.

Traditionally, reclamation has been carried out using agronomic grasses and legumes. The use of native woody plants having value for erosion control, watershed protection, production of browse and cover for wildlife, site amelioration, esthetics or timber production has potential in returning disturbed sites to greater biological and economic value.

The Alberta Forest Service, the Alberta Tree Nursery and Horticulture Center and the Reclamation Research Technical Advisory Committee are sponsoring a program to evaluate and select native woody species for use in reclamation in the area. The ultimate operational use of any plant species in part depends upon the feasibility of large-scale propagation. Previous work reviewed the seed pre-germination treatments required by 25 candidate species (King 1980). Subsequently, the needs of those species were further appraised and a seed-testing research project designed for those woody plants identified as difficult to grow from seed (King 1981). These problem species are listed in Table 1.

This report presents the results of the preliminary phase of the seed-testing research project.

Table 1

NATIVE WOODY PLANT SPECIES INVESTIGATED IN SEED PRE-GERMINATION TREATMENT TESTING (PRELIMINARY PHASE)

Scientific Name	Common Name(s)
Amelanchier alnifolia Nutt.	saskatoon, serviceberry, juneberry, shadbush
Arctostaphylos uva-ursi (L.) Spreng.	bearberry, kinnikinnik
Elaeagnus commutata Bernh.	silverberry, wolf willow
Juniperus communis L.	common juniper
Rosa acicularis Lindl.	prickly rose
Rosa woodsii Lindl.	Fendler woods rose, common wild rose, woods rose
Rubus parviflorus Nutt.	thimbleberry
Rubus strigosus Michx.	wild red raspberry
Shepherdia canadensis (L.) Nutt.	Canadian buffalo berry, russet buffalo berry, soapberry

SOURCE: Nomenclature follows that of Moss 1959.

2. ELAEAGNUS COMMUTATA SEED PRE-GERMINATION TREATMENT TEST

2.1 Introduction

Elaeagnus commutata Bernh. (silverberry, wolf willow) is a common deciduous shrub in Alberta of up to 4 m in height (Moss 1959). It is found throughout the province and is primarily a species of disturbed habitats (Moore 1964). Silverberry is an important nitrogen-fixing shrub (Vlassak et al. 1973; Bailey. 1973; Whysong and Bailey 1975). The species is considered to be a promising candidate for use in mined-land reclamation (King et al. 1982) and to date has performed well in oil sands reclamation trials.

The fruit of E. commutata is a dry indehiscent achene enclosed by a fleshy perianth (Olson 1974). The perianth is removed in seed cleaning while a thick stony pericarp remains on the seed. The species has a single seed per fruit (Corns and Schraa 1962).

2.2 Literature Review

The literature is contradictory on the existence and nature of seed dormancy and inconclusive on the optimum pre-germination treatment for breaking dormancy. Heit (1968) and Simonson (1976) reported that the seed was non-dormant and required no treatment to obtain germination. Shoemaker and Hargrave (1936) described the species as dormant due to hard seed while Heit (1968) concluded that hard seed coats did not occur in E. commutata and that scarification was not necessary. Others recommended cold stratification for 10 to 90 days as a necessary treatment to obtain moderate levels of germination (Babb 1959; Olson 1974; USDA 1979; Dick 1979). The last suggests endogenous dormancy but does not account for its mechanism.

Vories (1981) noted the possible presence of a germination inhibitor in the seed. Earlier work found that the inhibition was associated with the pericarp and concluded that these inhibiting substances exerted the dominant influence on the ability of silverberry seeds to germinate (Corns and Schraa 1962). This study also documented the unidentified inhibitor in the pericarp as water soluble.

2.3 Methods and Materials

Mature silverberry fruit was collected October 11 and 12, 1980 at latitude 49°30'N, longitude 114°20' and an altitude of 1 448 m. After extraction and cleaning, the seed was stored in sealed containers at 6.2% moisture content and 0°C until testing commenced.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The factors used in the design were:

a) Cold running water leach for 0, 24, 48, and 96 hours
b) Cold stratification for 0, 15, 30, 45, and 60 days.

In the cold water leach treatments the pericarp and seed were continuously rinsed at a rate of approximately 0.5 L/minute in cold running tap water. Cold stratification was carried out at 5°C using horticultural peat with a pH of 5.8 as the stratification medium. In leaching - cold stratification combination treatments, leaching preceded cold stratification.

Prior to the application of treatments, each seed sample was surface-sterilized with calcium hypochlorite (4% available chlorine) and then thoroughly rinsed with distilled water. All seed germination testing was done on sterile moist blotting paper in presterilized closed petri dishes in a germinator. The germinator regime, that of Corns and Schraa (1962), was maintained at a constant 20°C without light. The germination test duration was 21

days with germination counts made every seven days. A seed considered as germinated in this experiment met the criteria for normal shrub seedlings of the Association of Official Seed Analysts (1978). All results are reported as real germination.

2.4 Results and Discussion

The total germination results obtained in this experiment are presented in Figure 1. Seeds which were not subjected to any treatment failed to germinate after three weeks. This may suggest that the lot tested was dormant. However, Simonson (1976) obtained 42-57% germination in untreated seeds after 49 days of germinator testing. Therefore, the germination rate of untreated silverberry seed was very low.

All of the water leaching treatments yielded greater levels of germination than any of the cold stratification or combined water leaching - cold stratification treatments while the maximum level of germination obtained in the experiment was in response to leaching for 96 hours. These results demonstrate that this lot of E. commutata did not have a water-impermeable seed coat and partially confirm the conclusion of Corns and Schraa (1962): water-soluble inhibitors (presumably in the pericarp) exert the dominant influence on the germinability of the seed. The presence of water-soluble germination inhibitors has also been documented in Oryza sativa (Mikkelsen and Sinah 1961), Iris hybrids (Arditti and Pray 1969), Astragalus lentiginosus (Ziemkiewizc and Cronin 1981), Viburnum trilobum (Knowles and Zalik 1958) and Atriplex gardneri (Ansley and Abernathy 1982.)

In the leaching treatments the level of germination was proportional to the duration of the leaching. Greater levels of germination could likely have been attained by leaching the seed for a longer period or through the use of a greater leaching rate.

Cold stratification treatments resulted in only 2-7% germination. It should be noted that these relatively low germination levels may not accurately reflect the ultimate germinability of cold stratified seed. Greater total germination

has been reported for seed which had been cold - stratified for comparable periods of time but tested for 50 days under an alternating 20/30°C temperature regime (Olson 1974). However, the pronounced response differences obtained here between the leached and stratified seeds suggest the former would be more efficacious in any event.

The combined water leaching - cold stratification treatments gave no or relatively little germination. None appear to have potential as seed pre-germination treatments.

The experimental data for cumulative germination are presented in Figure 2. The greatest amount of germination generally occurred during the third and final week of the testing period and it was uncertain these germinative levels represented the peak germination rate. This indicates the duration of the seed tests may not have often been sufficient. In future work the germination testing period should be lengthened.

FIGURE 1. TOTAL GERMINATION IN *ELAEAGNUS COMMUTATA* SEEDS

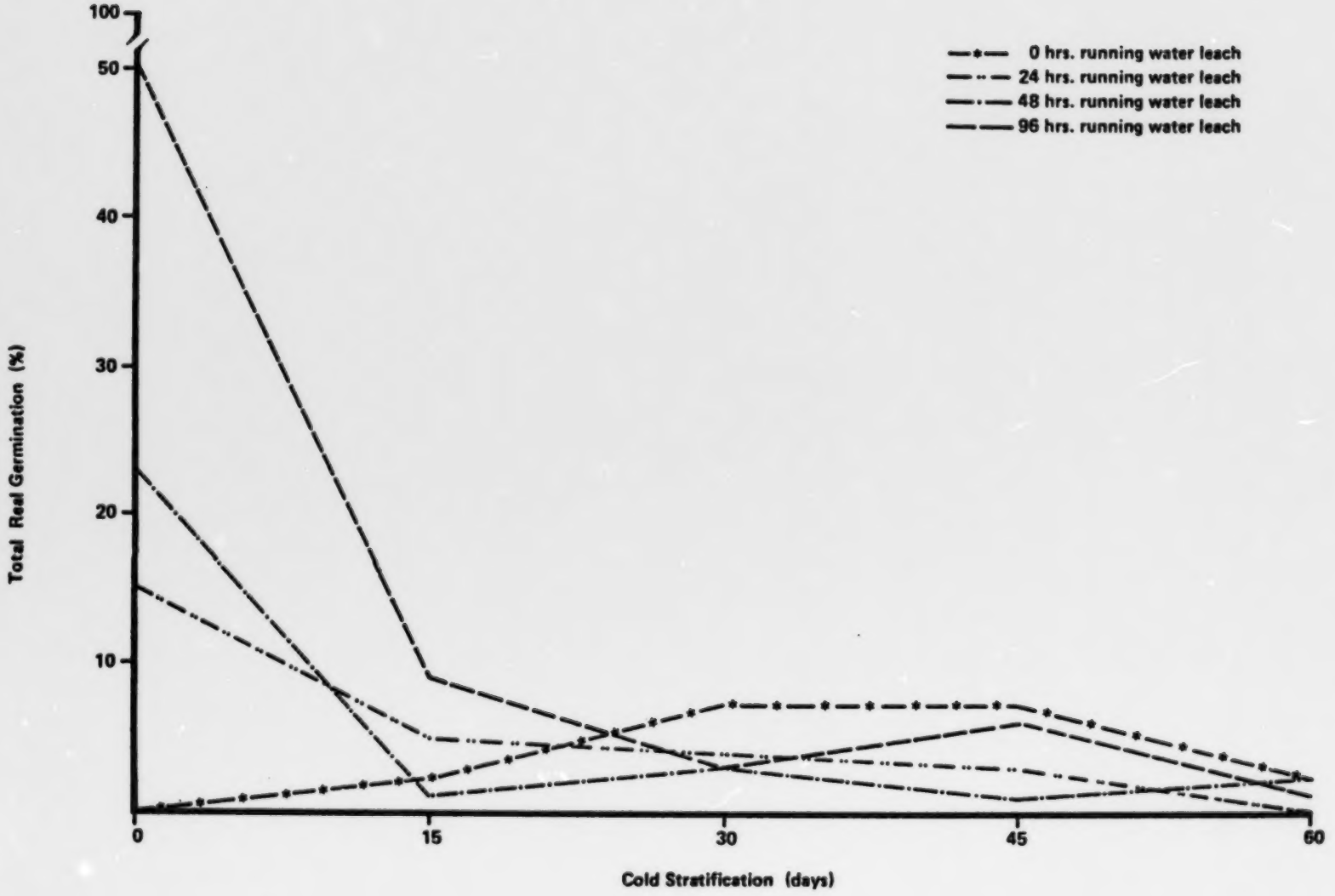


FIGURE 2. CUMULATIVE GERMINATION IN *ELAEAGNUS COMMUTATA* SEEDS

a) 0 Hrs. Running Water Leach

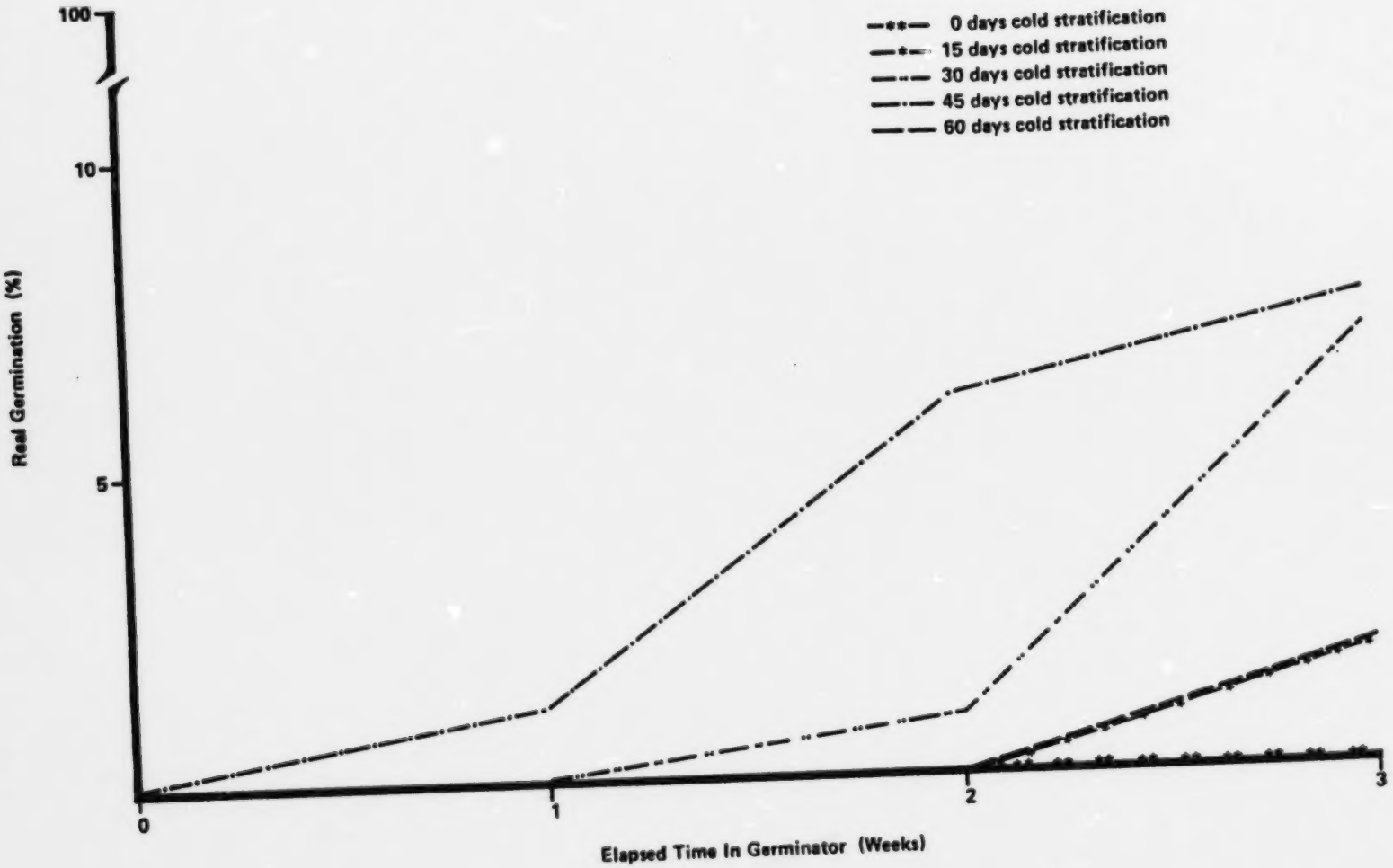


FIGURE 2. CUMULATIVE GERMINATION IN *ELAEAGNUS COMMUTATA* SEEDS

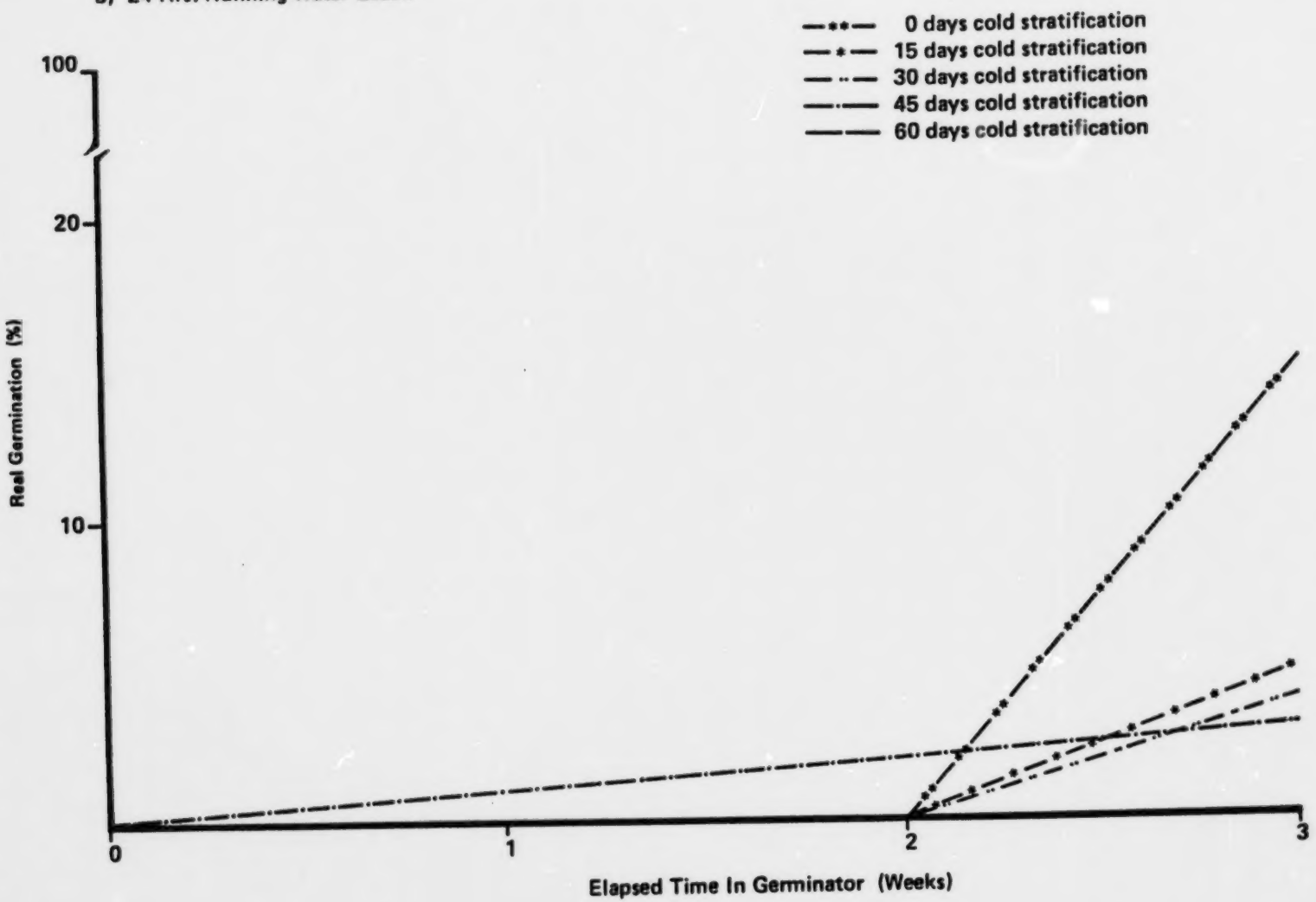


FIGURE 2. CUMULATIVE GERMINATION IN *ELAEAGNUS COMMUTATA* SEEDS

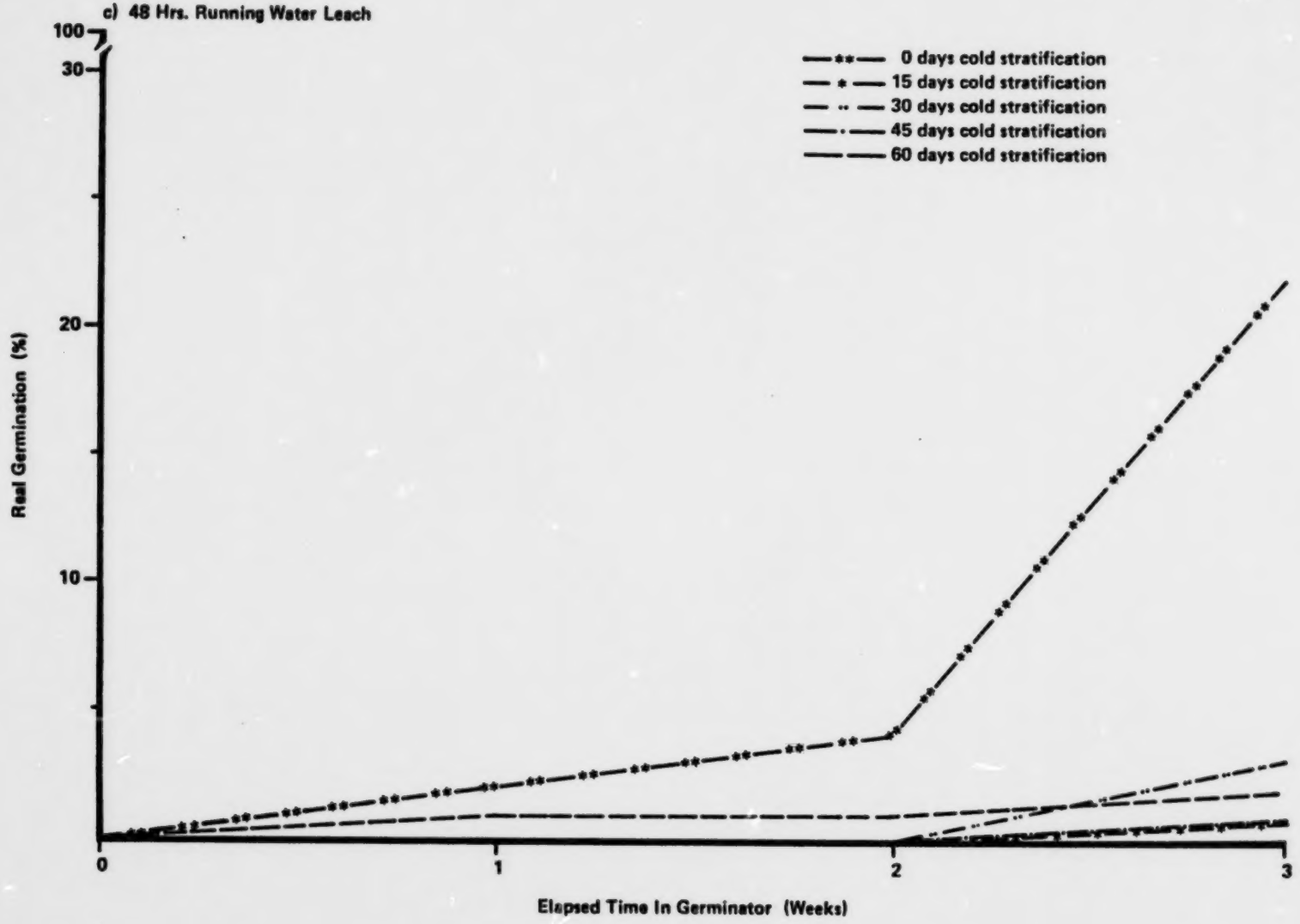
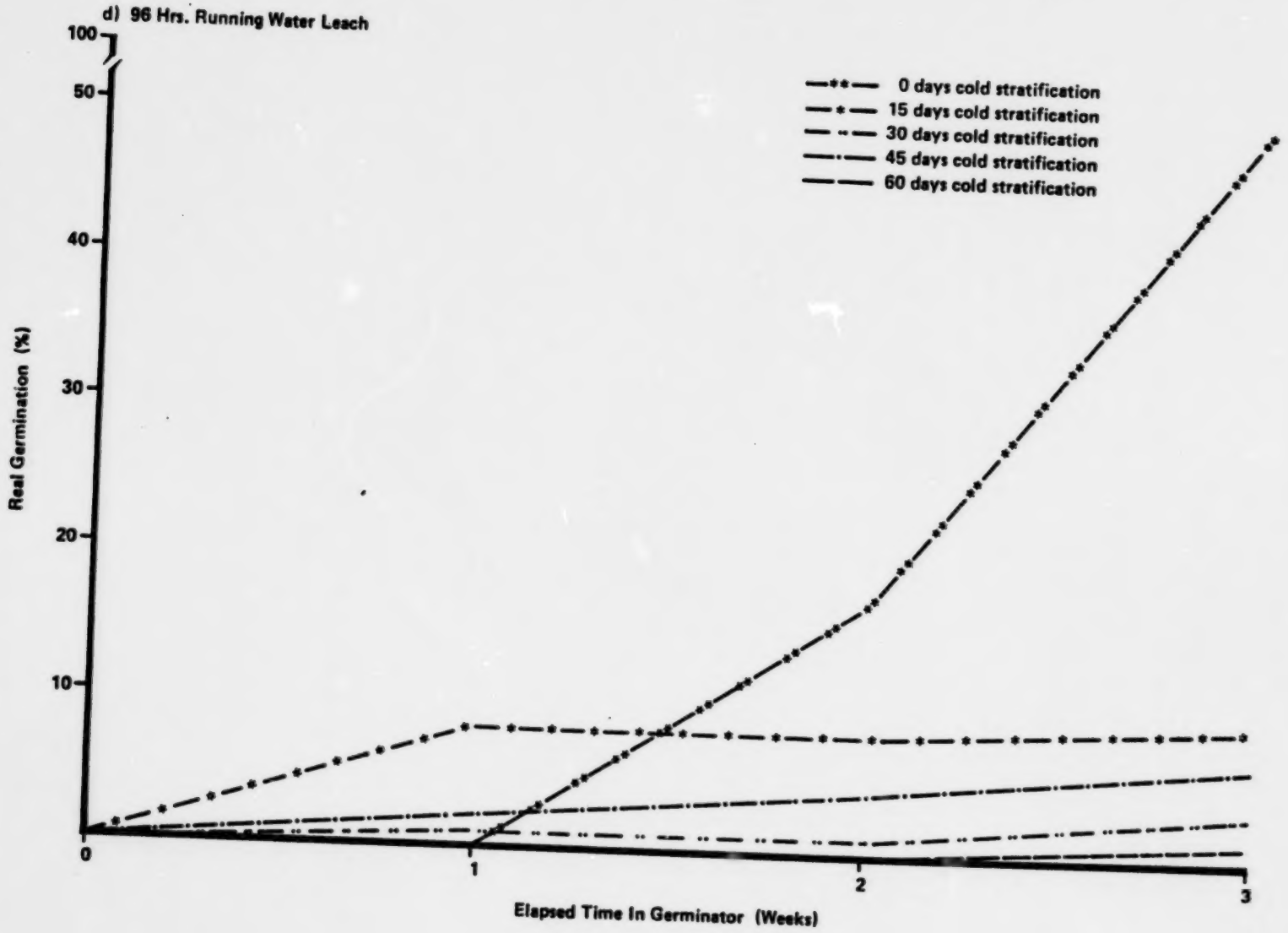


FIGURE 2. CUMULATIVE GERMINATION IN *ELAEAGNUS COMMUTATA* SEEDS



3. ROSA ACICULARIS
SEED PRE-GERMINATION
TREATMENT TEST

3.1 Introduction

Rosa acicularis Lindl. (prickly rose) is a common deciduous shrub in Alberta which is 0.5 - 2.5 m in height (Moss 1959). It is prevalently a species of the boreal zone (Densmore and Zasada 1977) but also occurs in the prairie, foothills and montane regions (Campbell et al. 1966; Taylor 1973). Prickly rose is highly adapated to disturbance (Plummer 1976; Russell 1979) and is a promising species for reclamation.

The fruit of the rose is an achene, several to many of which are borne within a fleshy berrylike hip (Gill and Pogge 1974). The rose propagule consists of an embryo enclosed within a thin testa and surrounded by a thick sutured pericarp (Densmore and Zasada 1977).

3.2 Literature Review

There is little authoritative information in the literature on the propagation of the species from seed. Babb (1959) recommended that the seed be scarified with sulphuric acid for one to two hours and then cold-stratified at 5°C for 75 days but did not report any work on which the recommendation was based. A pre-germination treatment of gibberellin followed by warm stratification and a subsequent cold stratification or a simple cold stratification treatment was similarly recommended without substantiating data (USDA 1979; DenHeyer et al., undated).

Research carried out in Alaska by Densmore (1974) and Densmore and Zasada (1977) forms the basis of documentation on the

species. These studies found that a dual stratification treatment (warm stratification for 118 days followed by 90 days of cold stratification) yielded the most complete germination. In contrast, treatment with cold stratification alone gave relatively low levels of germination. The authors felt that the dual stratification in R. acicularis fulfilled a role suggested by Nikolaeva (1969) and Villiers (1972): in the seed of some species warm temperatures are required to initiate the requisite metabolic process for physiological after-ripening while further stages continue only under cooler conditions.

Based on subsidiary studies, they reported that the presence of the testa and the physiological condition of the embryo were the major causes of dormancy in the species while the pericarp played a minor, if any, role in the prevention of germination. They also found the seed coverings were not impermeable and that scarification was not a beneficial pre-germination treatment. Some of these conclusions are notable as they differ from the reported findings for other Rosa species (Jackson and Blundell 1963; Gill and Pogge 1974).

The role of fruit and seed ripeness in the dormancy of Rosa is not well documented in the literature. Hartmann and Kester (1975) recommended that rose hips be collected as soon as they are ripe and still firm but did not cite any documentation to support such a recommendation. The role of germination inhibitors and promoters in seed dormancy has been studied in Rosa rugosa (Svejda and Poapst 1972) and in other members of the Rosaceae (Pollock and Olney 1959; Lin and Boe 1972). These studies dealt only with the relative changes in the concentrations of these substances during after-ripening but found that germination was dependent upon the levels of the former decreasing while the latter remained constant or increased. In some roses the level of inhibitors in the fruit, seed coverings and embryo is related to the stage of fruit and seed development (Hartmann and Kester 1975). In R. canina the effect of inhibitors in ripe fruit tissue is so pronounced that achenes left in the hips remain dormant in response to dual warm and cold stratification which produces substantial germination in free

achenes (Rowley 1956). Therefore, it was hypothesized that fruit ripeness at the time of seed collection and extraction play an important role in the germinability of R. acicularis seed.

3.3 Methods and Materials

Prickly rose hips were collected August 1, 1980 at latitude 53°22'N, longitude 117°45' and an altitude of 1 250 m. Two ripeness phases were gathered. In the first, referred to as "not fully ripe", the hips were yellowish-orange in color and their texture was firm. The second, designated as "fully ripe", had deep red color and firm textured hips. The latter corresponds approximately with the recommended ripeness for collection from Hartmann and Kester (1975). In both the seeds were in the hard dough stage. After extraction and cleaning the seed lots were dried to 6.0 - 6.2% moisture content and then stored in sealed containers at 0°C.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The factors used in the design were:

a) ripeness (as described previously)
b) warm stratification for 0, 60, 90, and 120 days.

The warm and cold stratification temperatures were 25° and 5°C respectively. When a dual stratification treatment was used, warm stratification was followed by cold. Moist horticultural peat moss with a pH of 5.8 was used as the stratification medium.

Prior to the application of stratification treatments, each seed sample was surface-sterilized with calcium hypochlorite (4% available chlorine) and then thoroughly rinsed with distilled water. All seed germination testing was done on sterile vermiculite in pre-sterilized closed petri dishes placed in a germinator. The germinator conditions were designed after those of Densmore and Zasada (1977) and were a constant 20°C with 16 hours of light per day. The germination test duration was 84 days with germination

counts made every seventh day. A seed considered as germinated in this experiment met the criteria for normal shrub seedlings of the Association of Official Seed Analysts (1978). All results are reported as real germination.

3.4 Results and Discussion

The total and cumulative germination results obtained in the experiment are presented in Figures 3 and 4 respectively.

Germination in unstratified seed was not appreciable. Cold statification alone gave relatively incomplete germination, although total germination was generally proportional to the duration of the cold stratification treatment.

The maximum levels of germination across ripeness treatments were obtained in response to 60 days of warm stratification followed by 120 days of cold stratification. The use of longer periods of warm stratification suppressed germination levels commensurately with the duration of warm stratification. This is markedly different from the results obtained by Densmore (1974) and Densmore and Zasada (1977) who obtained maximum germination after 118 days of warm stratification (followed by cold stratification). The warm moist conditions of warm stratification enhance bacterial and fungal activity (Giersbach 1937; Nikolaeva 1969; Pellett 1973) and, therefore, the decrease in germination in this experiment with the 90 and 120-day warm stratification treatments may be attributed to microbial damage of the embryo. The results for this lot of R. acicularis indicate that warm stratification may play a role of chemical breakdown in the dormancy-causing exterior seed layer(s) as has been documented in R. rugosa (Svejda and Poapst 1972). However, a possible function in breaking physiological embryonic dormancy as suggested by Densmore (1974) and Densmore and Zasada (1977) cannot be discounted.

The magnitude of some of the germination levels is not large when compared to those obtained by Densmore (1974) and Densmore and Zasada (1977). It is possible that this may be partially attributed to the annual variability in seed germinability. In collections

from the Banff area, it was noted that the germination obtained from the 1980 seed crop was lower than in other years (T. Laidlaw, personal communication). It has been demonstrated that climatic variability affects the germinability of cold stratified hybrid rose seed (Von Abrams and Hand 1956).

The ripeness treatment effects were significant in this experiment. The seed from the "not fully ripe" collection generally yielded greater germination than did the "fully ripe" treatment. This result suggests that hip ripeness at the time of collection and extraction affects the relative germinability of R. acicularis seed. Since many previous collections have been carried out in September or October (Dick 1979; Fedkenheuer and Heacock 1980; King, unpublished) the results may also indicate that collections should be carried out earlier than has been traditional.

Based on the results of the Alaskan studies cited previously the germination testing duration was designed for 12 weeks. Figure 4 demonstrates that most of the germination had occurred by the end of the fifth week. This may have application in future seed testing of the species.

FIGURE 3. TOTAL GERMINATION IN *ROSA ACICULARIS* SEED

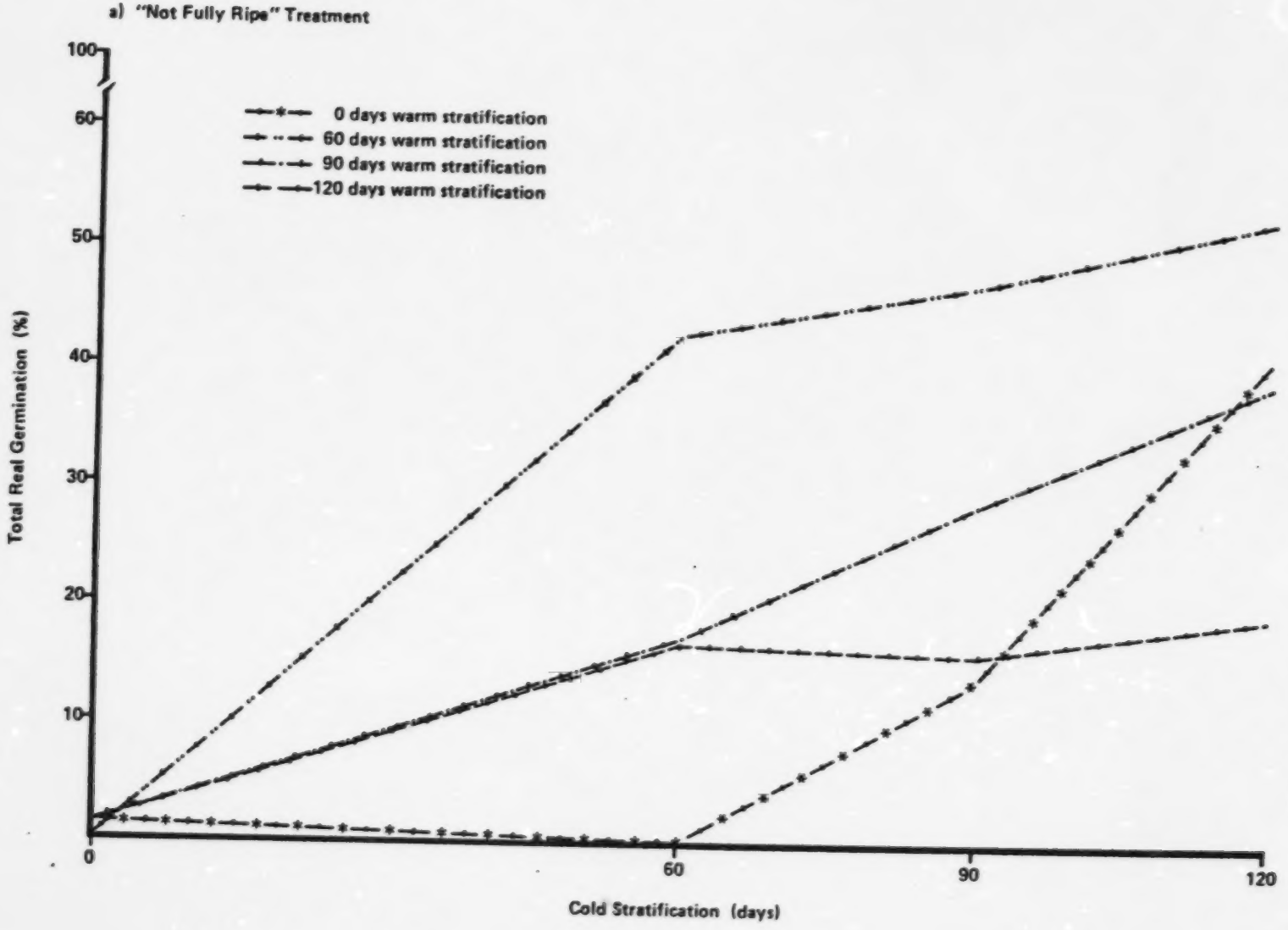


FIGURE 3. TOTAL GERMINATION IN *ROSA ACICULARIS* SEED

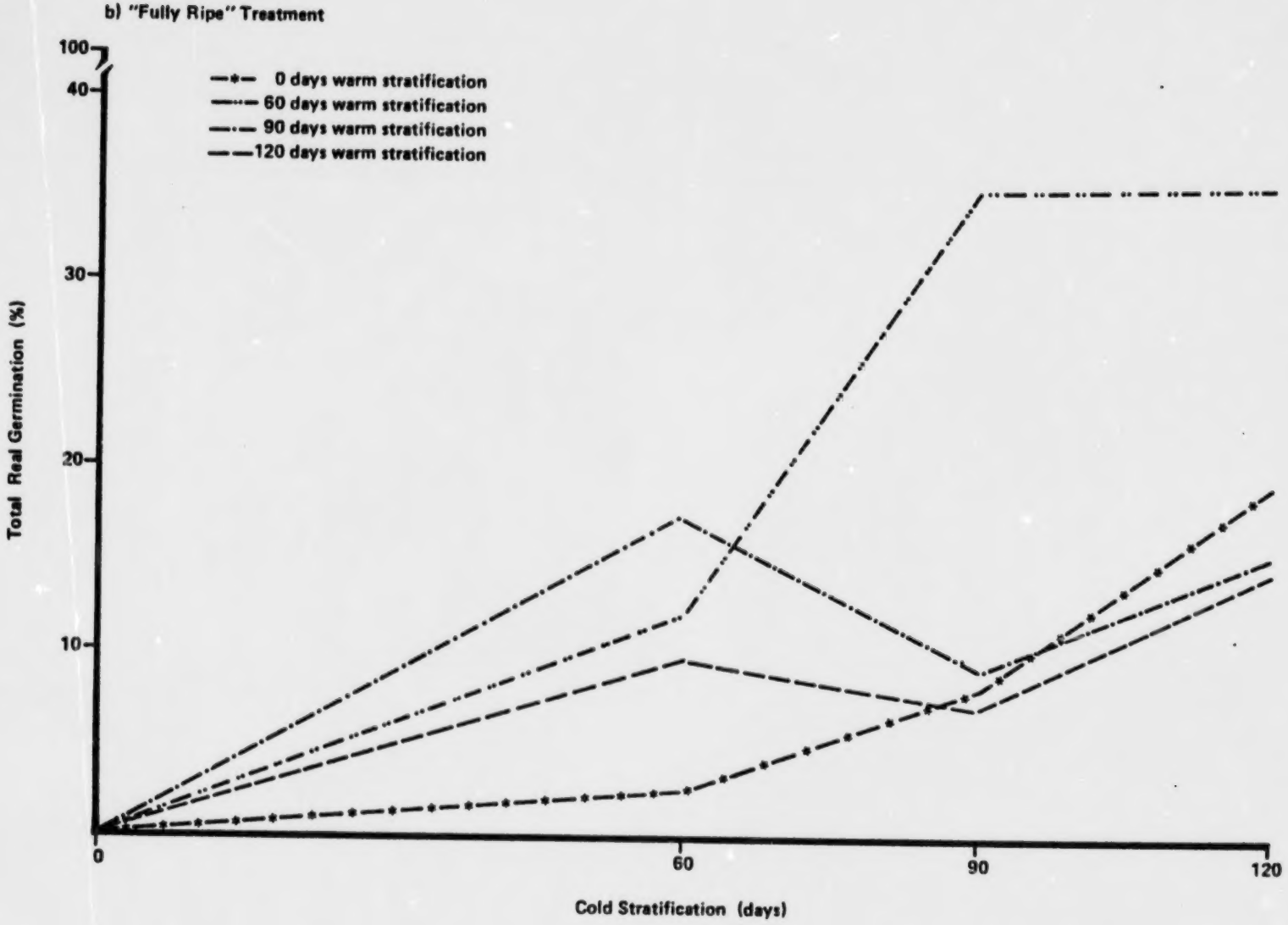


FIGURE 4. CUMULATIVE GERMINATION IN *ROSA ACICULARIS* SEED

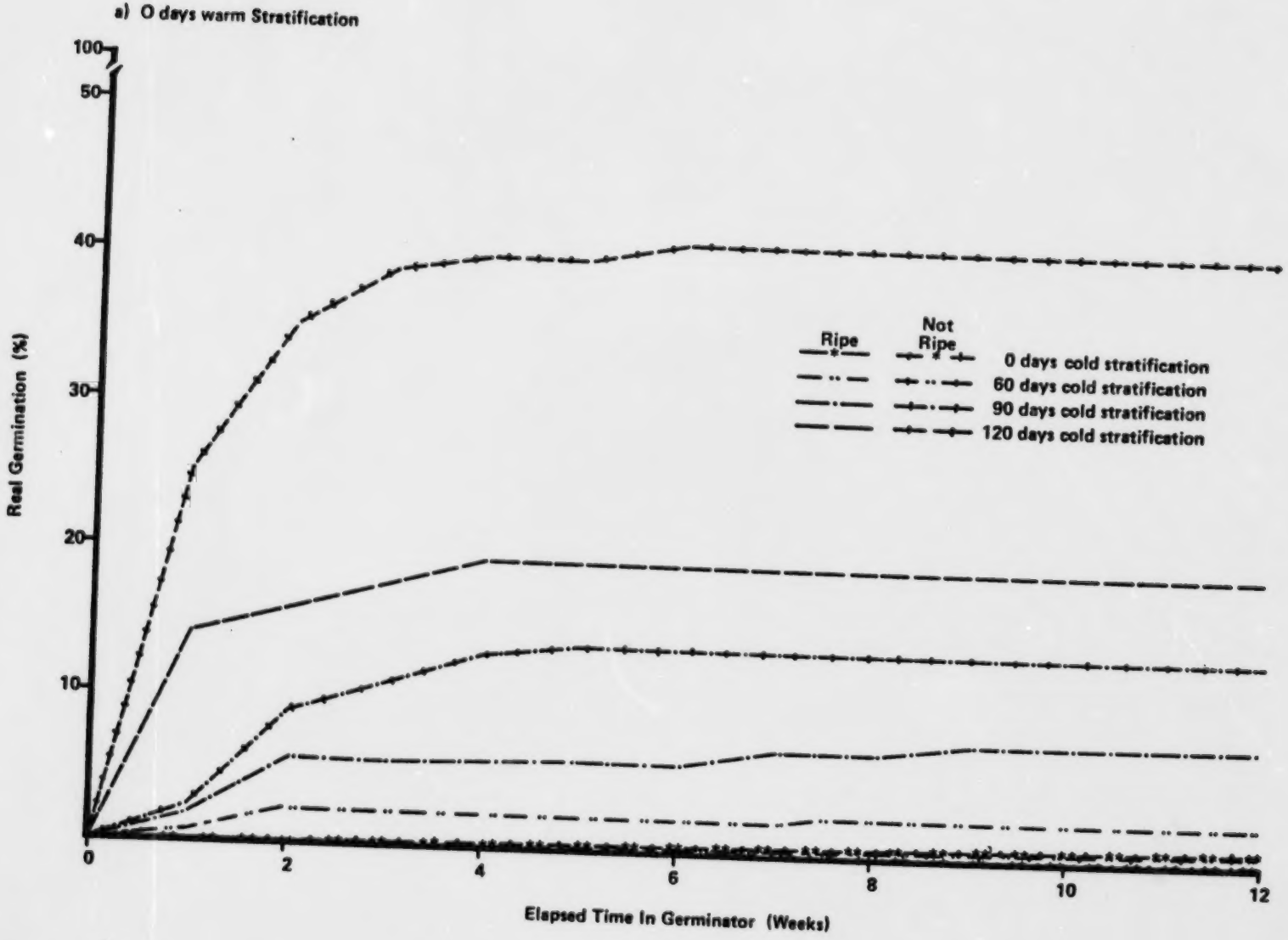


FIGURE 4. CUMULATIVE GERMINATION IN *ROSA ACICULARIS* SEED

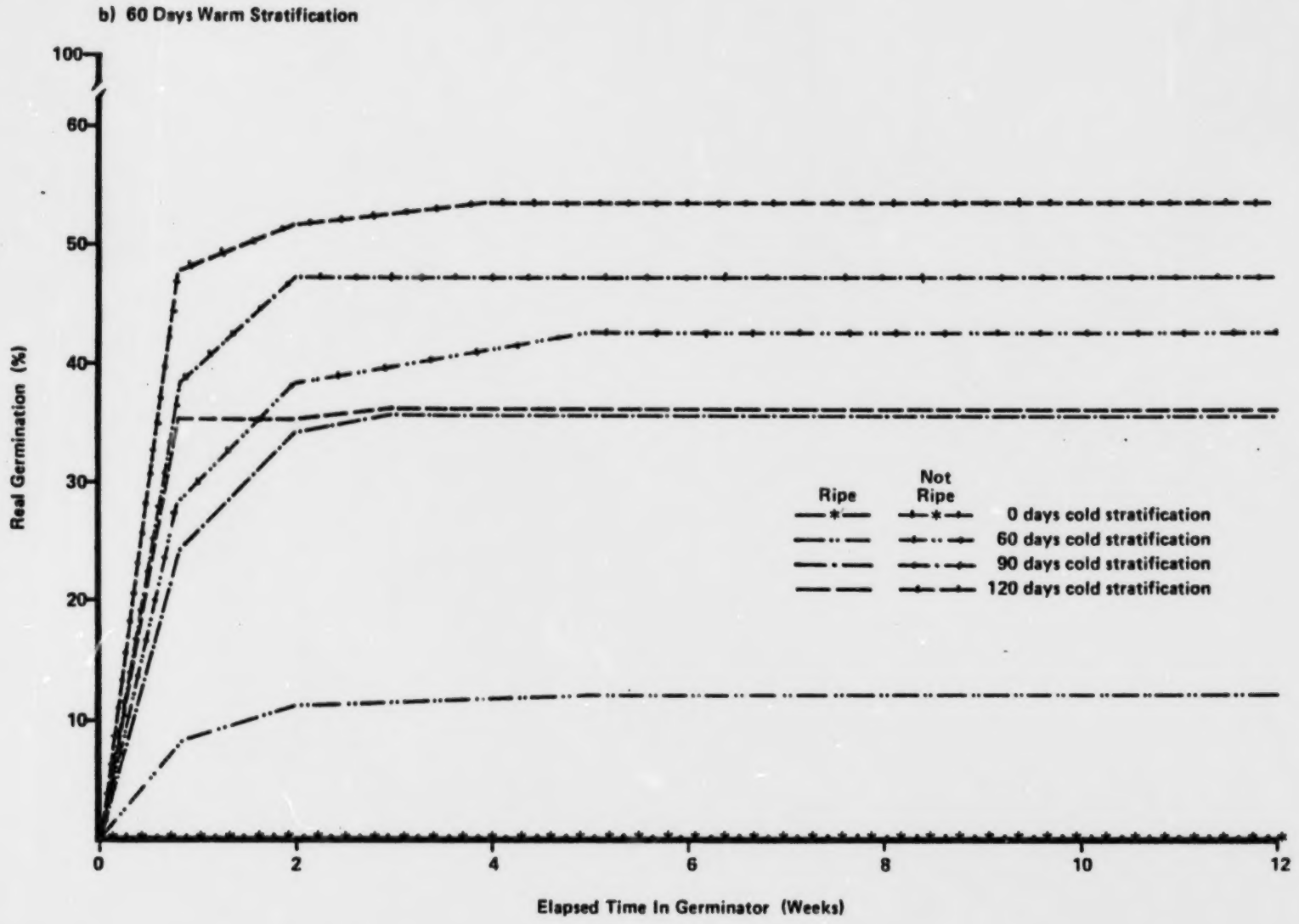


FIGURE 4. CUMULATIVE GERMINATION IN *ROSA ACICULARIS* SEED

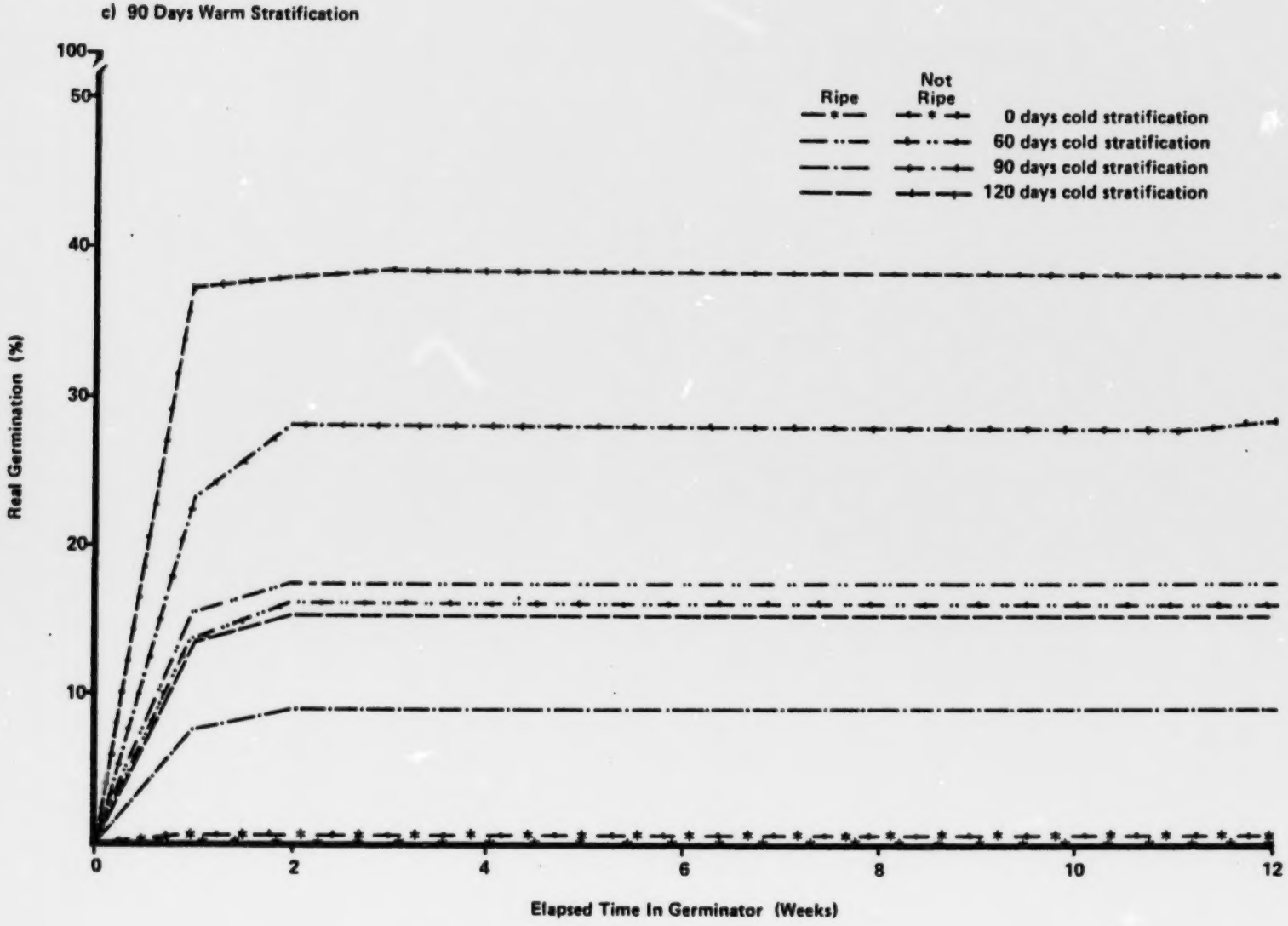
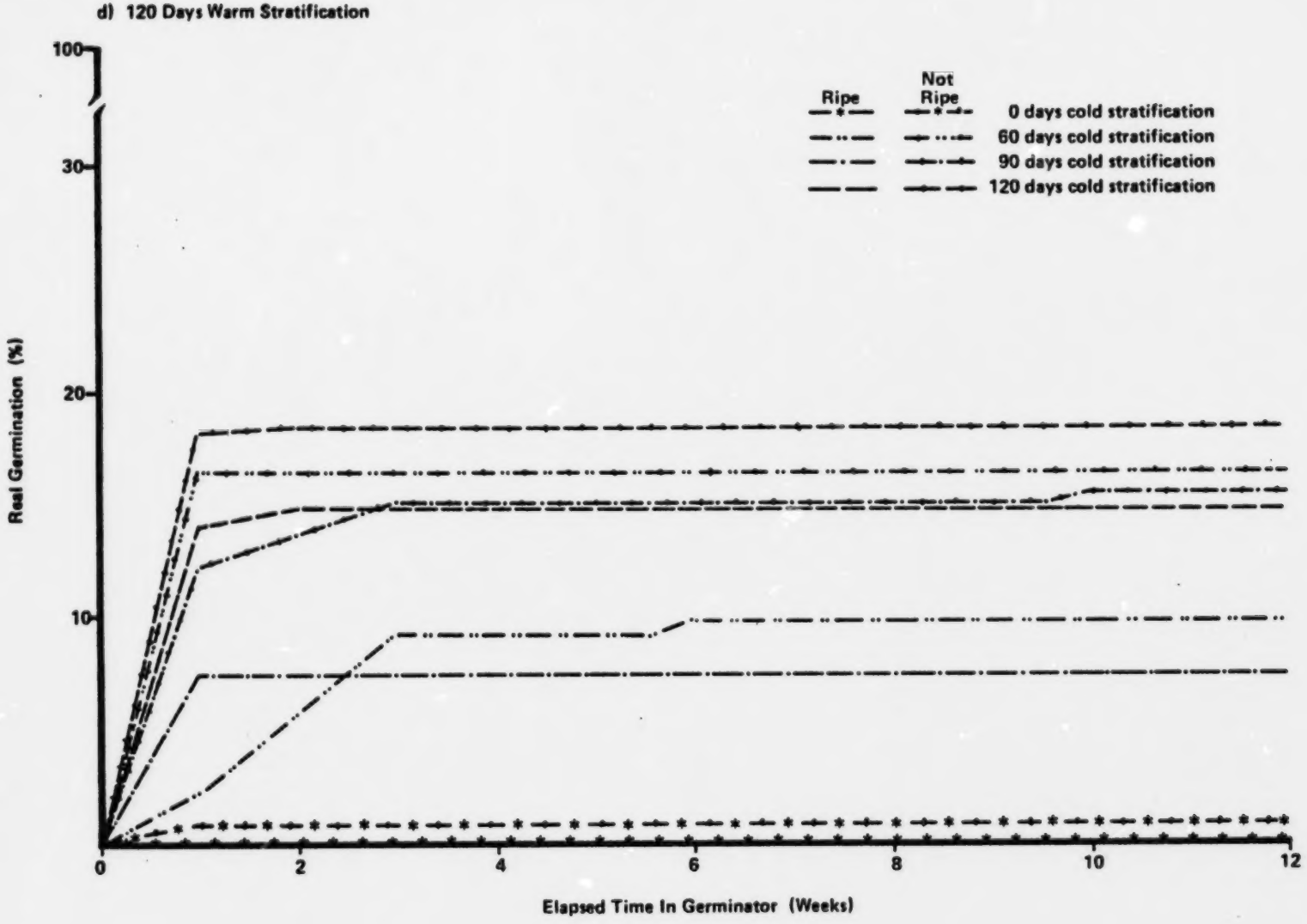


FIGURE 4. CUMULATIVE GERMINATION IN *ROSA ACICULARIS* SEED



4. ROSA WOODSII SEED PRE-GERMINATION TREATMENT TEST

4.1 Introduction

Rosa woodsii Lind. (woods rose, Fendler woods rose, common wild rose) is a tall deciduous shrub of 0.3 - 2.0 m in height (Moss 1959). A species of bluffs, dry slopes and sandhills in the prairies but also common on riverbanks and forest clearings in the boreal and subalpine regions (Watson et al. 1980), it is a species commonly associated with disturbance (Russell 1979) and has potential in reclamation of mined lands and soil stabilization (USDA, undated).

The fruit of the rose is an achene, several to many of which are borne within a fleshy berrylike hip (Gill and Pogge 1974). The rose propagule consists of an embryo enclosed within a thin testa and surrounded by a thick sutured pericarp (Densmore and Zasada 1977).

4.2 Literature Review

The literature on R. woodsii is inconclusive on the treatments required to break dormancy and contains no discussion on the dormancy mechanisms of the species. Only one reference reviewed presented germination yield data: 40-60% germination resulted from a dual stratification treatment (USDA 1979). This reported need for warm stratification followed by cold stratification agreed with the recommendations of Gill and Pogge (1974) and Dick (1979). Various other sources advised a simple cold stratification treatment at temperatures ranging from 0^{o} to $10^{o}C$ for periods as long as one year (Chadwick 1935; Swingle 1939; DenHeyer et al., undated). Stark (1966) recommended a dual treatment of concentrated sulphuric acid

followed by cold stratification although Heit (1967a) stated it is doubtful that seed of the genus Rosa would benefit from scarification.

Since there was no baseline study reported on woods rose, the intent of the experiment described herein was to identify possible effective seed pre-germination treatments. In addition, the possible effects of fruit and seed ripeness on germinability, as reviewed previously for Rosa acicularis, were studied.

4.3 Methods and Materials

R. woodsii hips were collected September 15, 1980 at latitude 53°55'N, longitude 119°15' and an altitude of 1 320 m. The description of the ripeness collections were as previously given for R. acicularis. After extraction and cleaning, the seed lots were dried to 6.0% moisture content and then stored in sealed containers at 0°C.

The information on experimental design and treatments, seed sterilization, and germination testing regime and methods were as in R. acicularis.

4.4 Results and Discussion

The total and cumulative germination results obtained in this experiment are presented in Figures 5 and 6 respectively.

Seeds which were not stratified did not germinate. The use of warm stratification alone did not yield appreciable levels of germination. Simple cold stratification treatments gave relatively incomplete seed germination, although total germination was proportional to the duration of the cold stratification.

The maximum levels of germination across ripeness treatments were obtained in response to 60 days of warm stratification followed by 90 or 120 days of cold stratification. Both the duration of this dual pre-germination treatment and the level of germination obtained generally agree with that reported by the Native Shrub Production Project in the western United States (USDA 1979). In the experiment

herein, the use of warm stratification for periods exceeding 60 days (as the first part of a dual stratification treatment) suppressed germination levels commensurably with the duration of the warm stratification. A similar trend was observed in R. acicularis and, as discussed under that species, the decrease in germination with the longer warm stratification periods may be due to microbial damage to the embryo.

The ripeness treatment effects were significant at the 95% level in the experiment. The seeds from the "not fully ripe" treatment generally yielded greater germination than did the "fully ripe". As discussed under R. acicularis, this may ultimately have practical as well as biological benefit.

The 12-week germination test duration used in this experiment was based on that of prickly rose from Densmore (1974) and Densmore and Zasada (1977). Figure 6 demonstrates that most of the germination had occurred by the end of the fifth week. This may have application in future seed testing of this species.

FIGURE 5. TOTAL GERMINATION IN *ROSA WOODSII* SEED

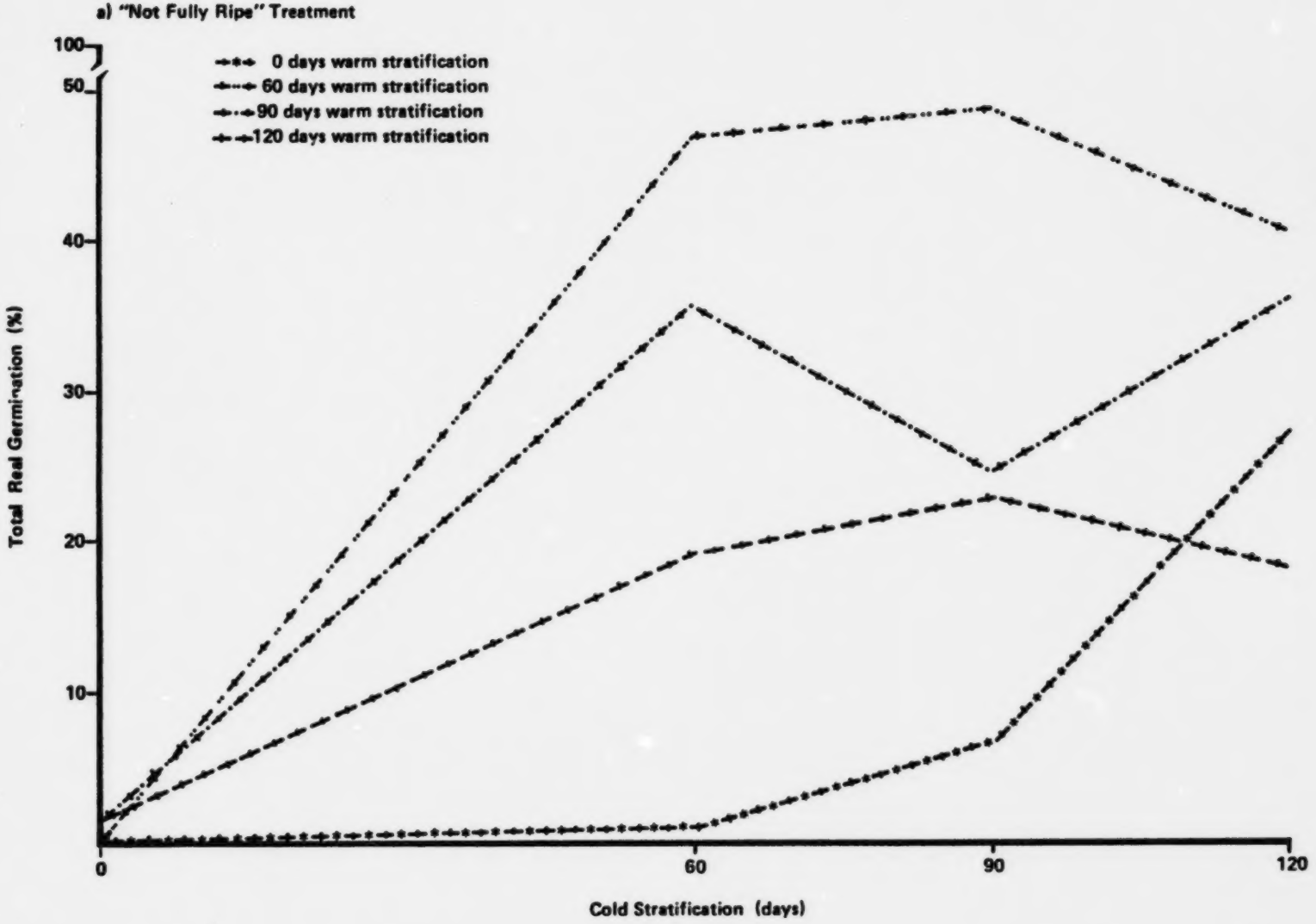


FIGURE 5. TOTAL GERMINATION IN *ROSA WOODSII* SEED

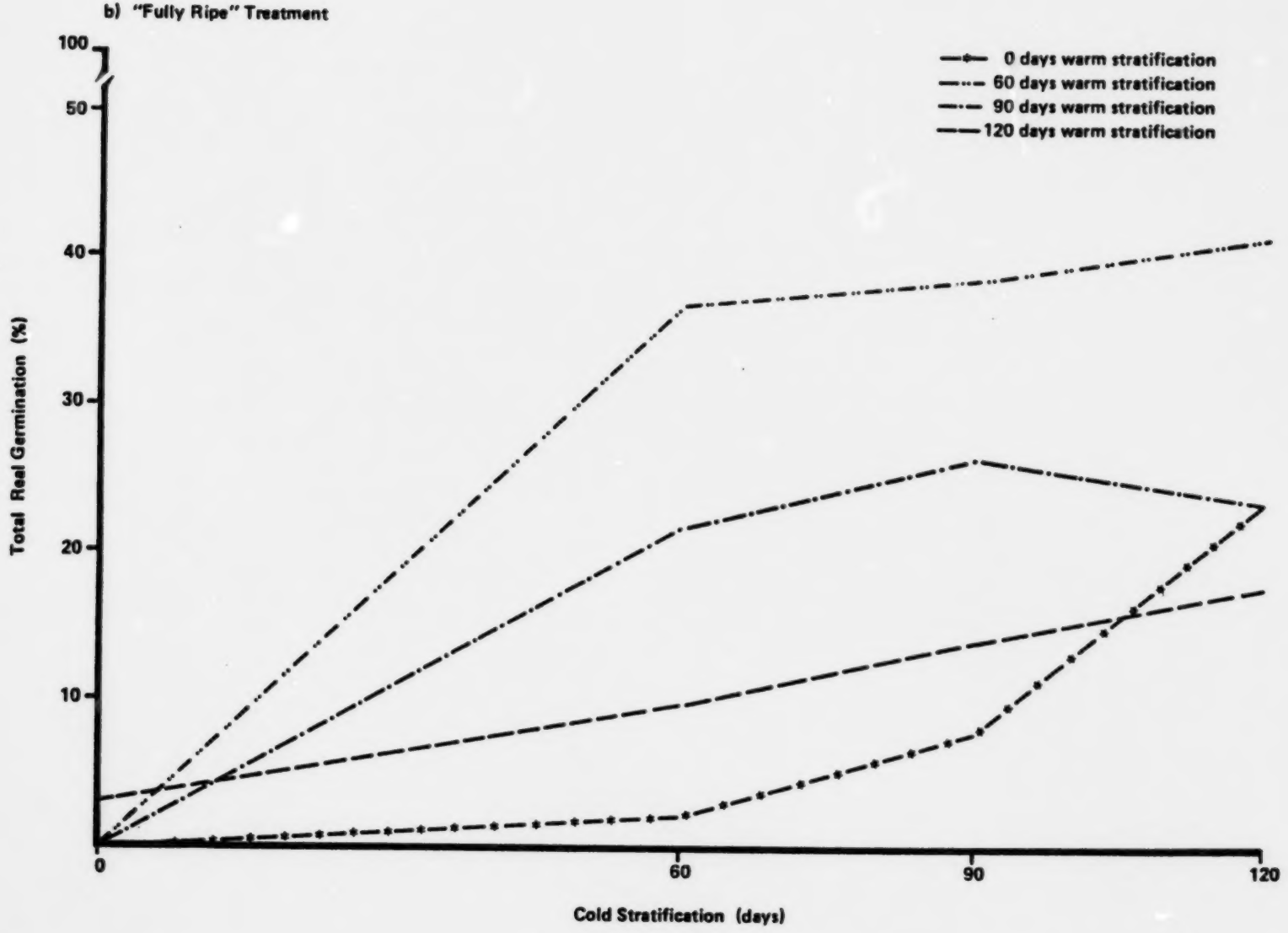


FIGURE 6. CUMULATIVE GERMINATION IN *ROSA WOODSII* SEED

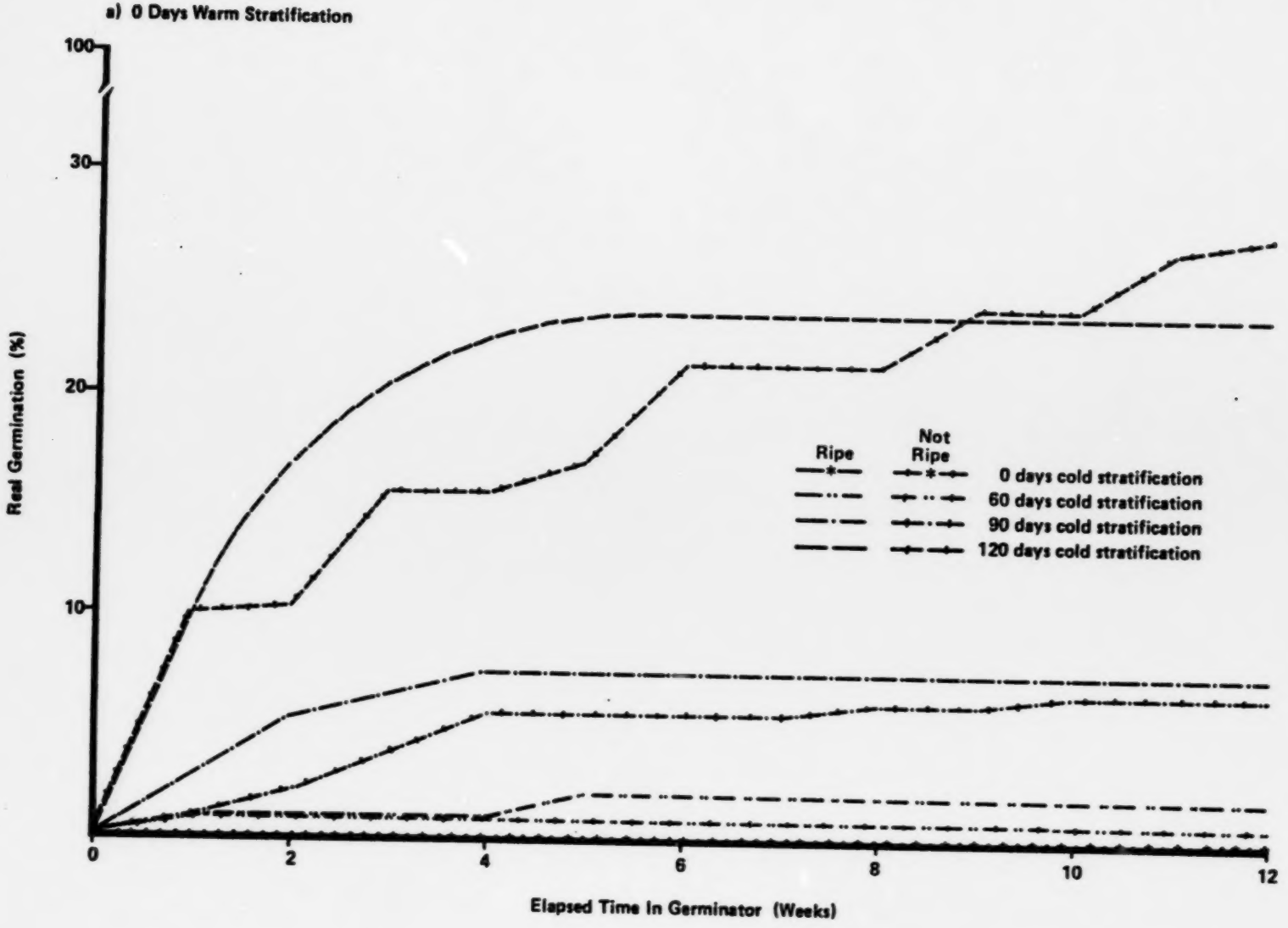


FIGURE 6. CUMULATIVE GERMINATION IN *ROSA WOODSII* SEED

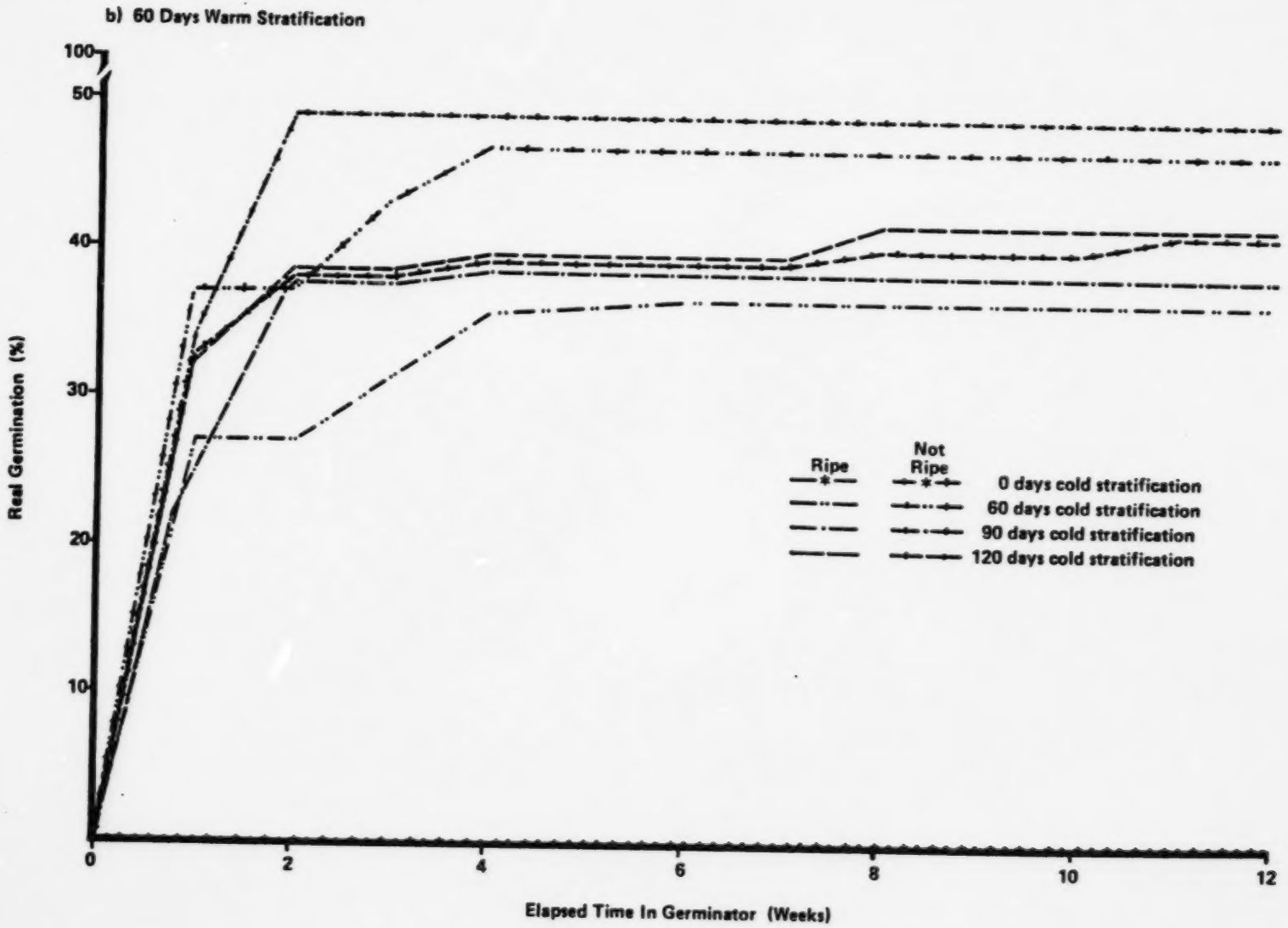


FIGURE 6. CUMULATIVE GERMINATION IN *ROSA WOODSII* SEED

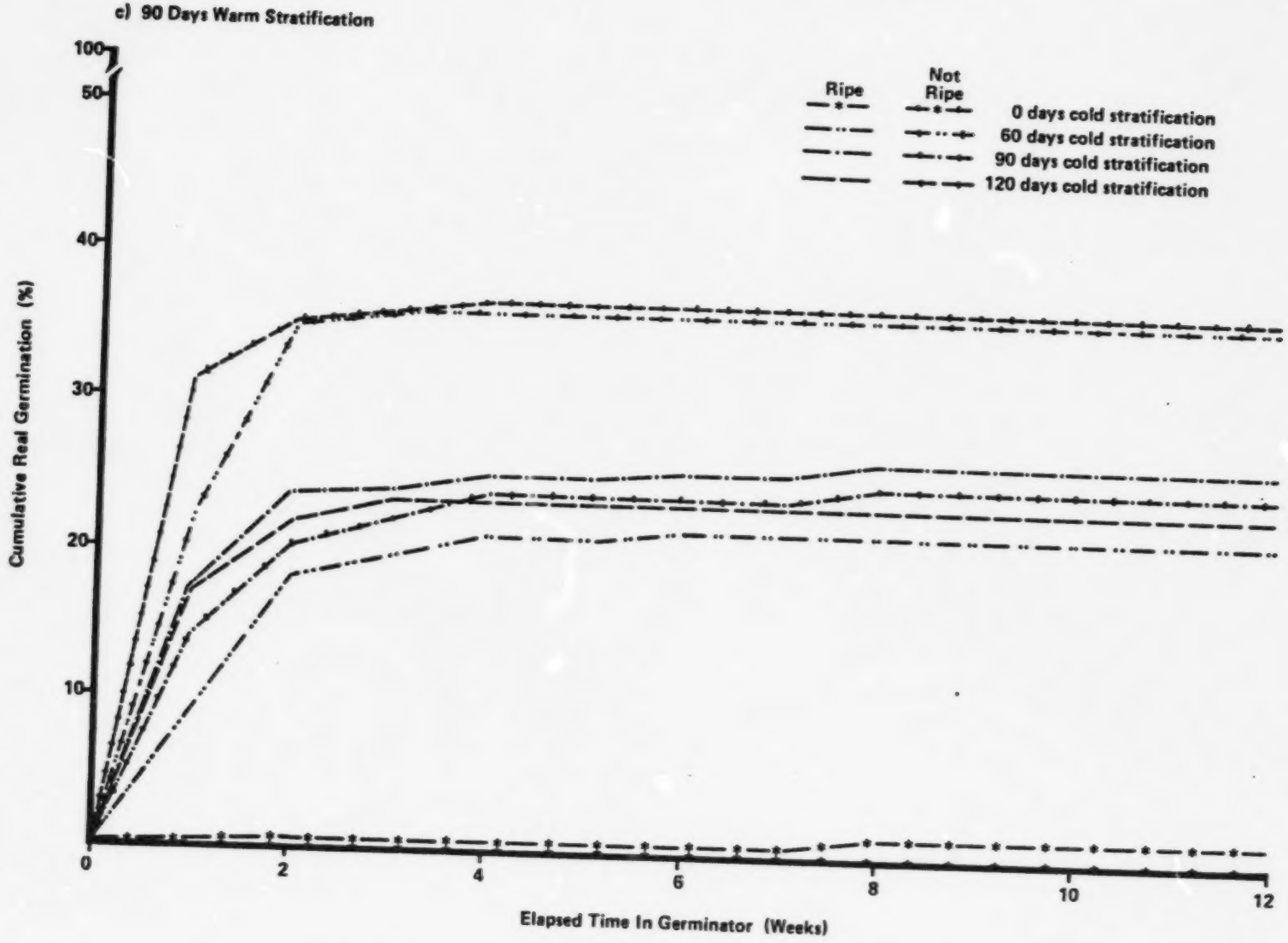
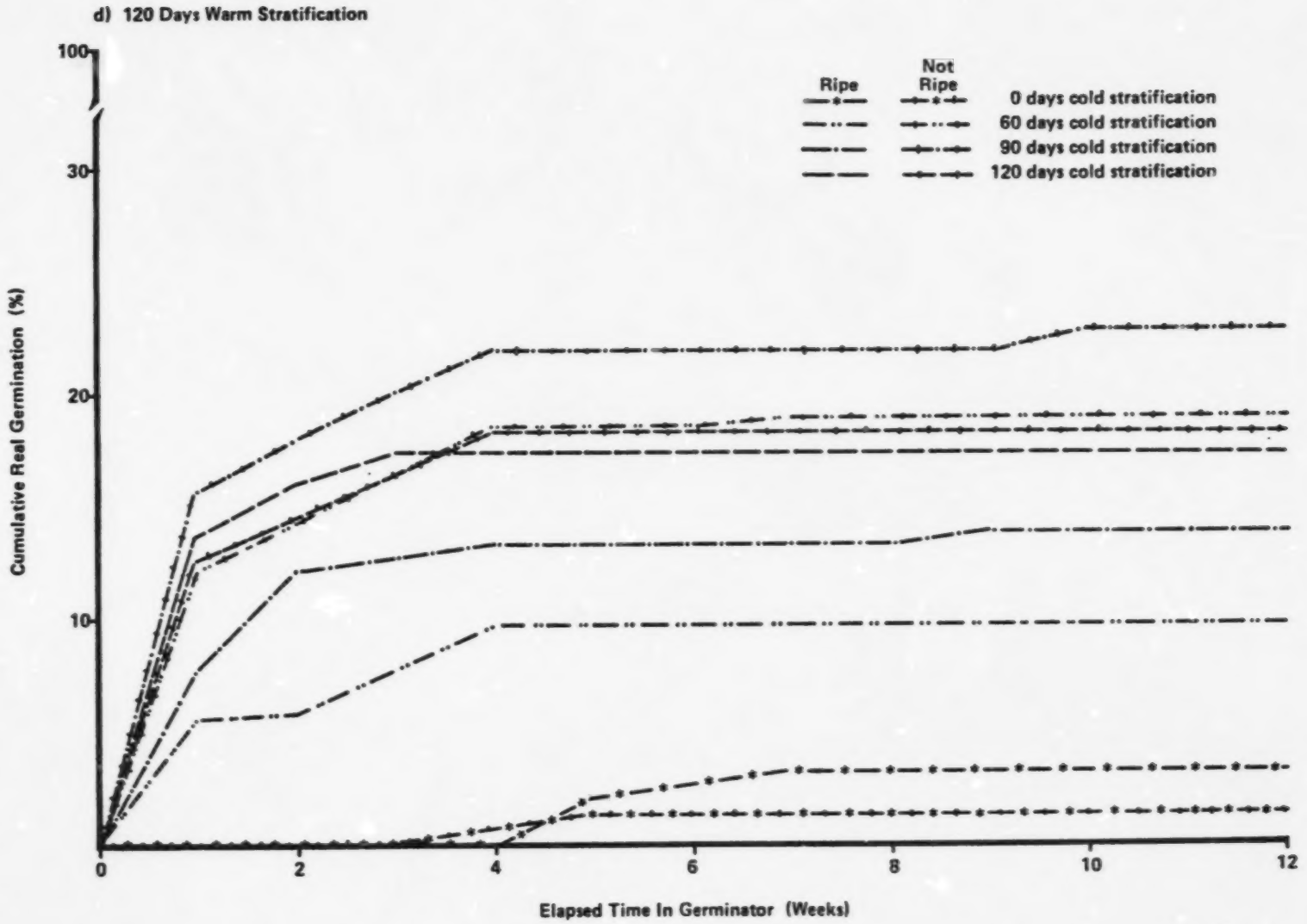


FIGURE 6. CUMULATIVE GERMINATION IN *ROSA WOODSII* SEED



5. RUBUS PARVIFLORUS
SEED PRE-GERMINATION
TREATMENT TEST

5.1 Introduction

Rubus parviflorus Nutt. (thimbleberry) is an erect shrub, 0.5 - 2.0 m tall (Moss 1959). It is common in forest margins and openings in the Rocky Mountains of southwestern Alberta and rarer in the more northerly Rockies of the province (Looman and Best 1979). Occurrence was common on disturbed sites in the southern Rocky Mountain Eastern Slopes of Alberta (Russell 1979).

The fruit of Rubus is an aggregate of small succulent drupes, each of which contains a single hard-pitted nutlet (Brinkman 1974). The date of R. parviflorus fruit ripening is highly variable, occurring as early as June 30 and until September 3 in northern Idaho (Schmidt and Lotan 1980).

5.2 Literature Review

The literature on thimbleberry pre-germination treatments is virtually non-existent. The only reference found on the species was that of Marchant (1980). He reported "good" levels of germination with both 50 minutes of acid scarification and cold stratification at 2°C for six months.

The seed of many Rubus species has been reported to have an impermeable endocarp and to require scarification to break the dormancy thus caused (Heit 1967a; Brinkman 1974). Members of the genus also have a dormant embryo, and cold stratification or a dual treatment of warm stratification followed by cold stratification is needed (Ibid.).

5.3 Methods and Materials

Mature fruit was collected August 28, 1980 at latitude 49°30'N, longitude 114°20' and an altitude of 1 835 m. After extraction and cleaning, the seed was stored in sealed containers at 6.0% moisture content and 0°C until treatments commenced.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The factors used in the design were:

a) scarification (seeds were non-scarified or mechanically scarified)

b) warm stratification for 30, 60, 90, and 120 days

c) cold stratification for 60, 90, and 120 days.

Scarification was abrasive; sandpaper was used to remove a portion of the endocarp and testa without damaging the embryo. The warm and cold stratification temperatures were 25° and 5°C respectively. The stratification medium was horticultural peat with a pH of 5.8. The sequences of treatments used are illustrated in Figure 7. At the time of the germination test unstratified samples of unscarified and scarified seeds were also tested but not included in any statistical analysis.

FIGURE 7. TREATMENT COMBINATIONS USED IN RUBUS PARVIFLORUS TEST

SEED SAMPLES	Warm Stratification ---------- Cold Stratification
	Scarification-- Warm Stratification-- Cold Stratification

Prior to the application of seed treatments, each seed sample was surface-sterilized with calcium hypochlorite (4% available chlorine) and then thoroughly washed with distilled water. All seed germination testing was done on sterile sand in pre-sterilized closed petri dishes. The germinator was maintained at a 20/30°C alternating temperature with light for eight hours during the high temperature period. The germination test duration was 35 days with

germination counts made every seventh day. A seed considered as germinated in this experiment met the criteria for normal shrub seedlings of the Association of Official Seed Analysts (1978). All results are reported as real germination.

5.4 Results and Discussion

The total and cumulative germination results obtained in this experiment are presented in Figures 8 and 9 respectively.

Seeds which were not stratified germinated relatively incompletely (Table 2). In the experiment maximum germination was obtained in response to 30 or 60 days, of warm stratification followed by 90 or 60 days respectively of cold stratification in unscarified seed.

It should be noted that the cold stratification effect was not significant in the analysis and, therefore, that germination was independent of the duration of those cold stratification treatments used. However, the differences in germination between the unstratified samples and the experimental results suggest that cold stratification is likely a necessary pre-germination treatment (although this experiment did not establish a minimum cold requirement).

Table 2

GERMINATION IN UNSTRATIFIED
RUBUS PARVIFLORUS SAMPLES

Mechanical Scarification	Duration of Warm Stratification (Days)	Duration of Cold Stratification (Days)	Total Real Germination(%)
no	0	0	29
yes	0	0	16

Pre-germination treatment by mechanical scarification yielded less germination than was obtained in unscarified treatments. Scarification was not a beneficial treatment in this experiment. This result and the difference in germination between the non-scarified and scarified unstratified samples indicate that the physical attributes of the seed coverings are not likely a major cause of dormancy.

The effect of warm stratification was significant in the experiment. Greater germination was obtained in response to 30 or 60 days of warm treatment while the levels were generally suppressed by the longer periods (likely because of microbial damage to the embryo). In the literature warm stratification has been considered as playing various possible roles such as overcoming morphological dormancy (Heit 1971); physically breaking down hard or restrictive seed coverings (Giersbach 1937; Pellett 1973; Krugman et al. 1974); chemically breaking down germination inhibitors in seed coverings (Densmore 1974); or initiation of requisite germination metabolic processes (Nikolaeva 1969; Villiers 1972). In the R. parviflorus lot tested it is doubtful that the first two functions were of great consequence while either or both of the last two roles might have been of importance.

The germination testing regime used in this experiment was based on that reported by Brinkman (1974) for Rubus strigosus. These test conditions were effective for R. parviflorus.

FIGURE 8. TOTAL GERMINATION IN *RUBUS PARVIFLORUS* SEEDS

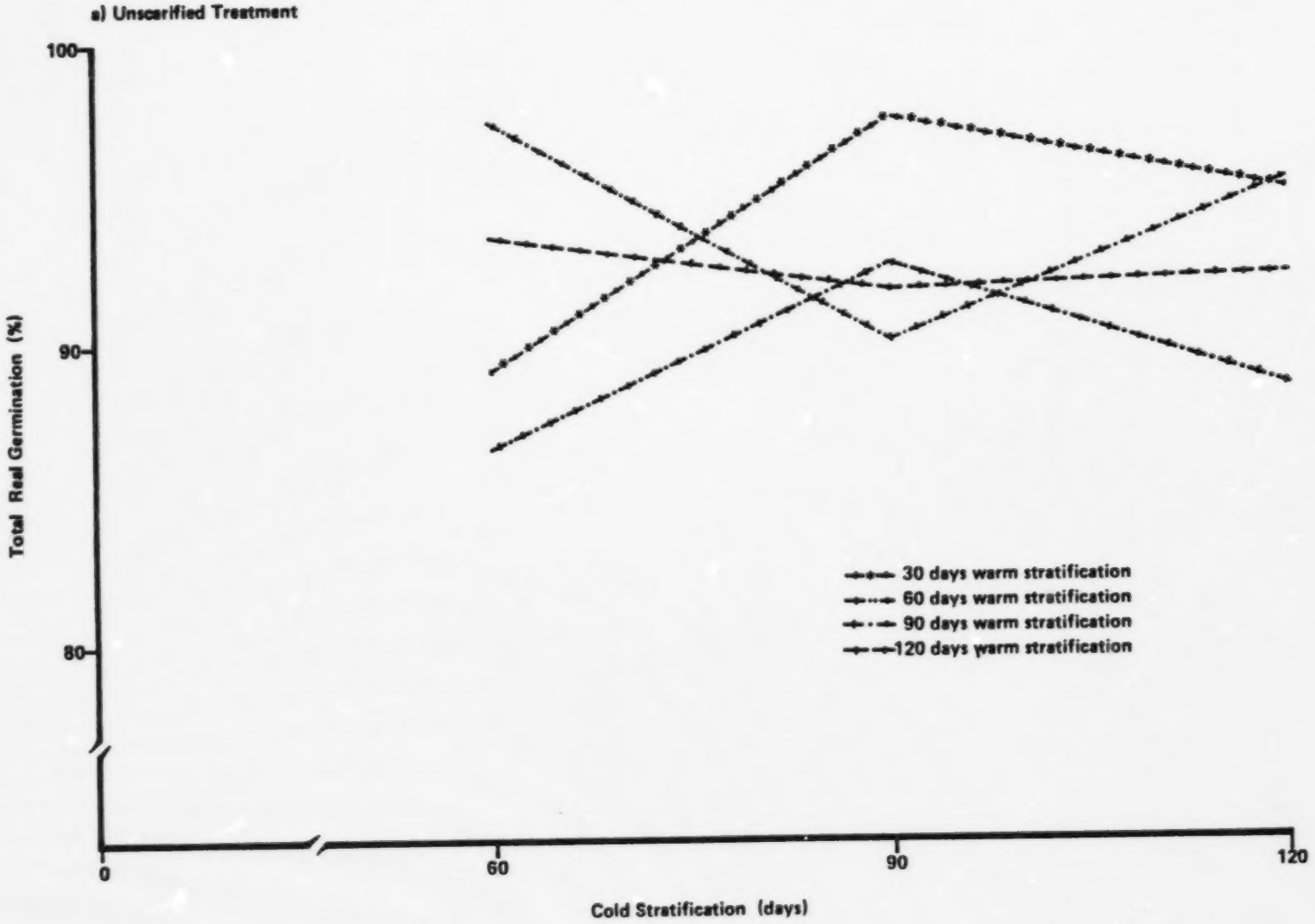


FIGURE 8. TOTAL GERMINATION IN *RUBUS PARVIFLORUS* SEEDS

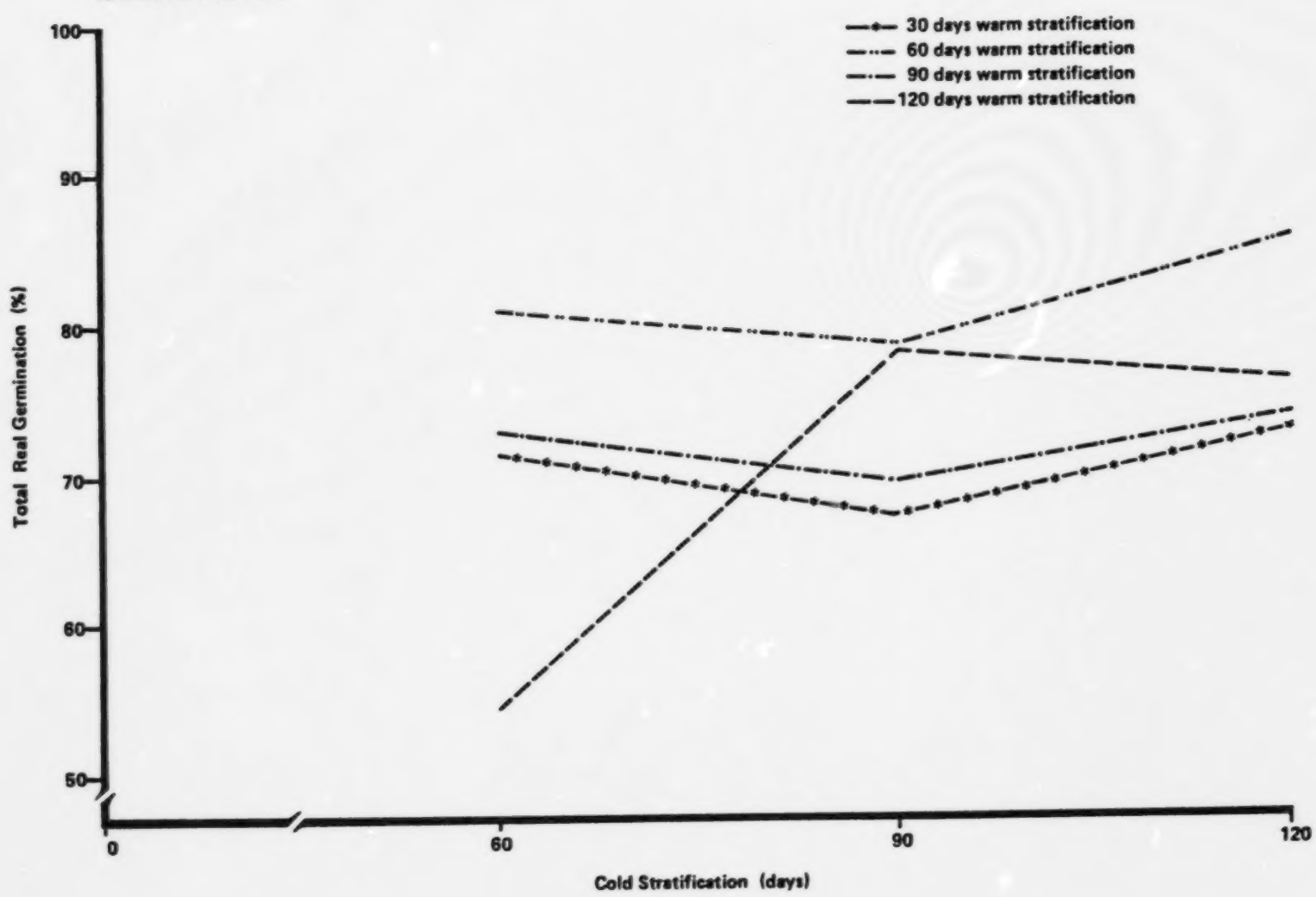


FIGURE 9. CUMULATIVE GERMINATION IN *RUBUS PARVIFLORUS* SEEDS

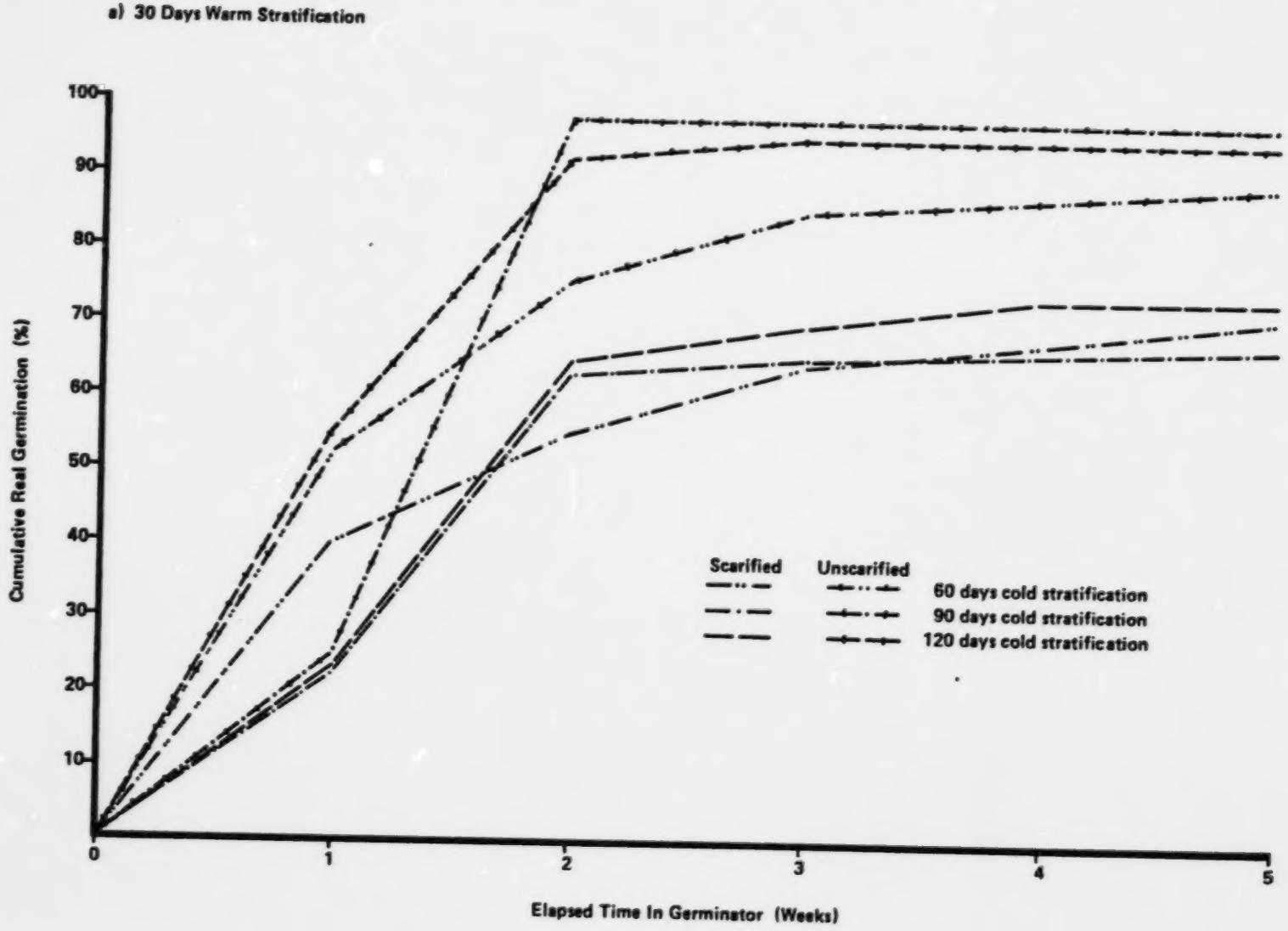


FIGURE 9. CUMULATIVE GERMINATION IN *RUBUS PARVIFLORUS* SEEDS

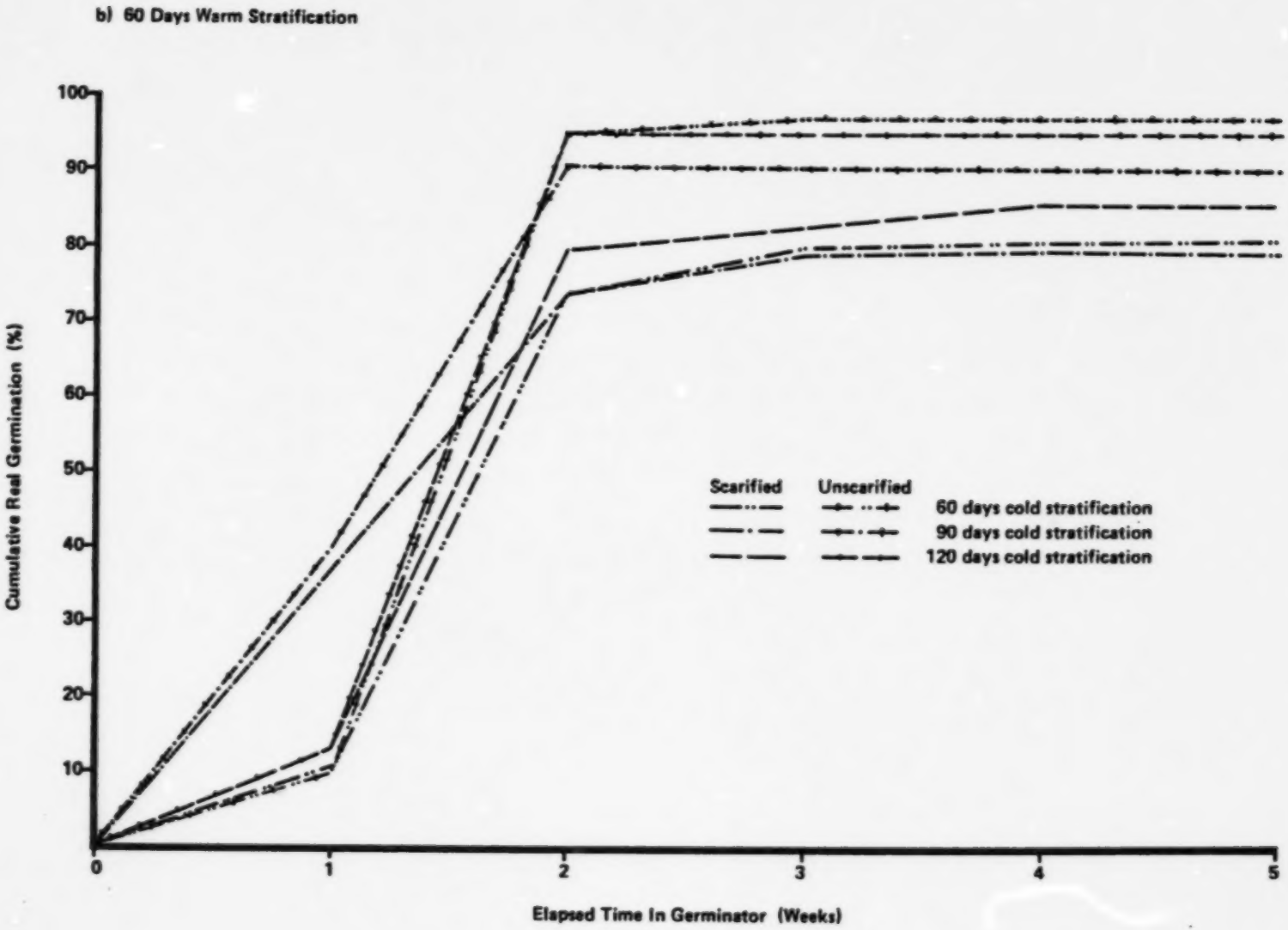


FIGURE 9. CUMULATIVE GERMINATION IN *RUBUS PARVIFLORUS* SEEDS

c) 90 Days Warm Stratification

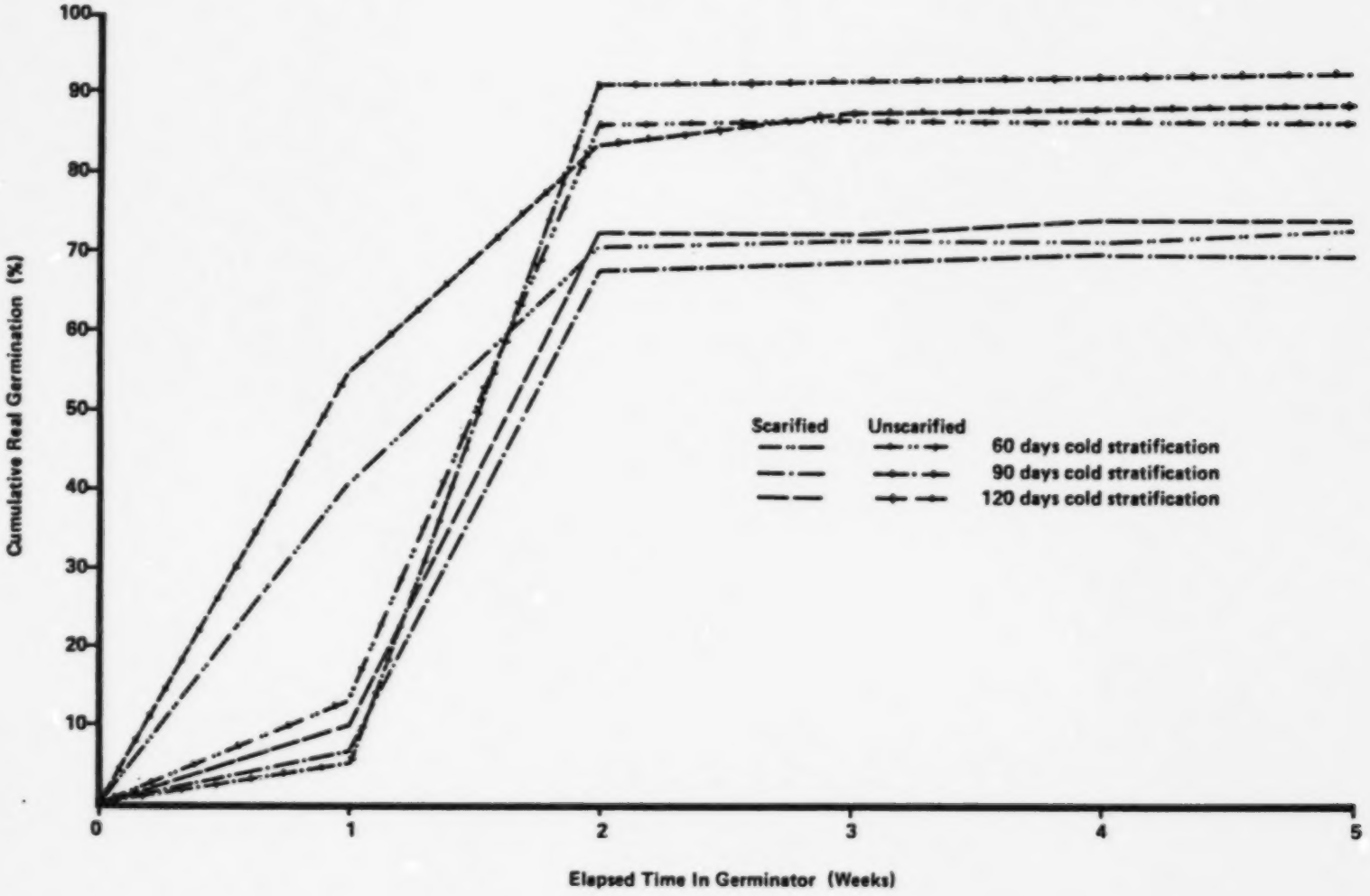
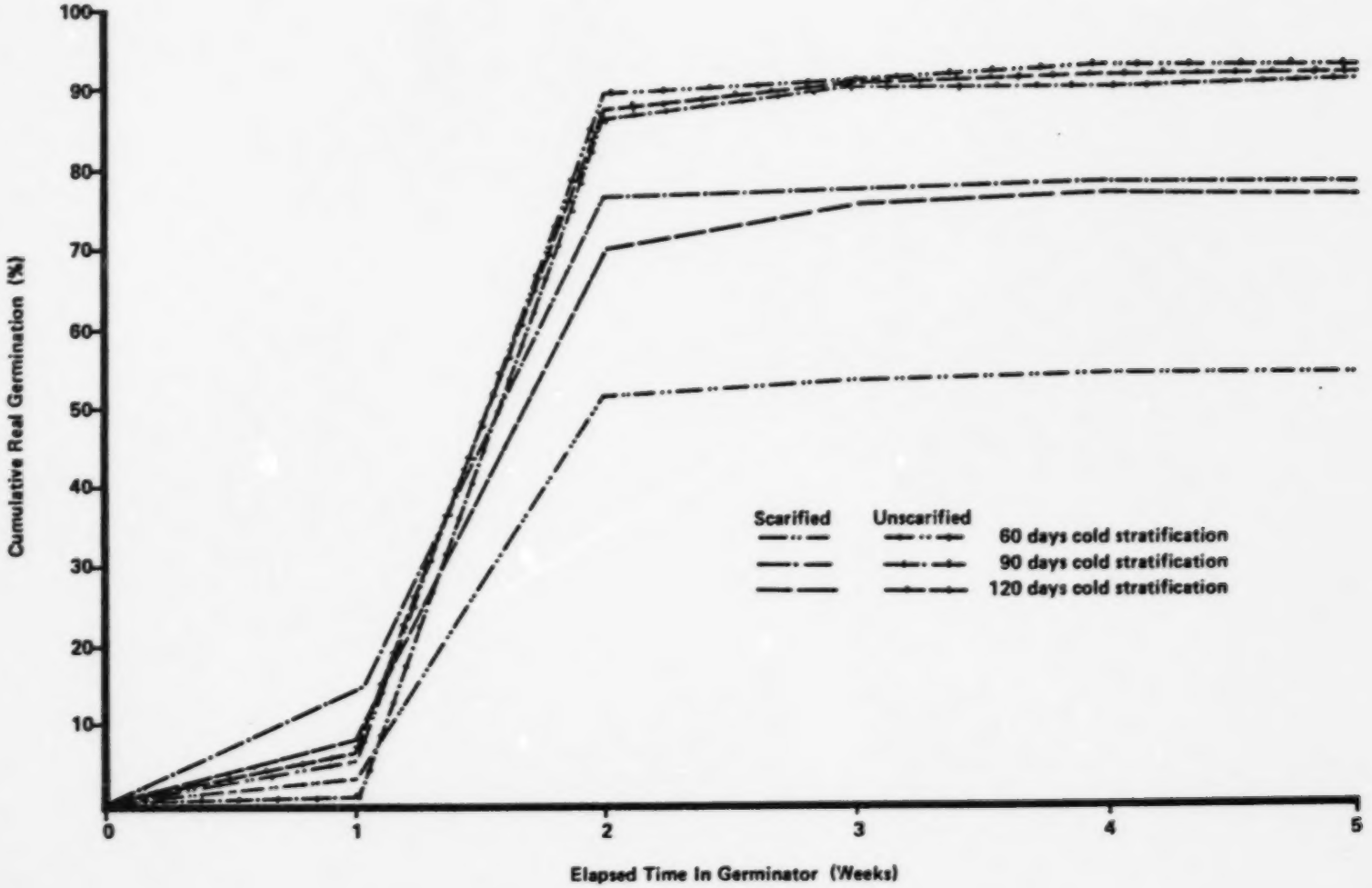


FIGURE 9. CUMULATIVE GERMINATION IN *RUBUS PARVIFLORUS* SEEDS

d) 120 Days Warm Stratification



6. RUBUS STRIGOSUS
SEED PRE-GERMINATION
TREATMENT TEST

6.1 Introduction

Rubus strigosus Michx. (wild red raspberry) is a deciduous bushy shrub 0.6 - 2.0 m in height (Moss 1959). It is common on land disturbances throughout the latitudinal range of the Eastern Slopes of the Rocky Mountains and extends from the lower foothills and montane zones into the subalpine (Russell 1979). The species is also common throughout the boreal forest in clearings, at edges of woods and on burned areas (Watson et al. 1980). It is a promising shrub species for use in reclamation.

The fruit of R. strigosus is an aggregate of small succulent drupes, each of which contains a single hard-pitted nutlet (Brinkman 1974).

6.2 Literature Review

Much of the available literature on pre-germination treatments of R. strigosus is composed of brief, sometimes contradictory recommendations with little or no supporting data. Heit (1967a) stated that the species was hardseeded while Afanasiev (1964) reported that it was not. Chadwick (1935) suggested that concentrated sulphuric acid scarification was a satisfactory treatment for overcoming seed dormancy. Cold stratification has also been recommended as an effective pre-germination treatment (Shoemaker and Hargrave 1936). Others have advised the use of dual acid scarification - cold stratification or warm stratification - cold stratification treatments (Babb 1959; Brinkman 1974; Heit 1967b).

An early study on the species remains one of the best documented in the literature. Rose (1919) found the failure of red raspberry seed to absorb water was not a limiting factor in germination since both entire seeds and excised embryos fully imbibed after five hours of water soaking. This study also reported that seed dormancy was associated entirely with the endocarp and/or testa since germination of excised embryos was rapid and complete without the application of any additional treatments. However, in his conclusions Rose hypothesized that dormancy may have been due to the high breaking strength of the endocarp, although no evidence was presented of such.

Scott and Ink (1957) reported a study on the effects of scarification, warm stratification and cold stratification on red raspberry seed germination. They found that warm stratification played a significant role in improving germination while triple scarification - cold stratification - warm stratification treatments gave maximum germination. However, their results also indicated that even with the triple treatment, germination levels of about only 50% could be expected after a four-month-long testing period.

6.3 Methods and Materials

Mature fruit was collected August 24, 1980 at latitude $52^{O}50'$N, longitude $116^{O}55'$ and an altitude of 1 430 m. After extraction and cleaning, the seed was stored in sealed containers at 6.0% moisture content and 0^{O}C until treatments commenced.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The factors used in the design were:

a) scarification (seeds were non-scarified or mechanically scarified)

b) warm stratification for 30, 60, 90, and 120 days

c) cold stratification for 60, 90, and 120 days.

Scarification was abrasive; sandpaper was used to remove a portion of the endocarp and testa without damaging the embryo. The

warm and cold stratification temperatures were 25° and 5°C respectively. The stratification medium was horticultural peat with a pH of 5.8. The sequences of treatments used are illustrated in Figure 10. At the time of the germination test unstratified samples of unscarified and scarified seeds were also tested but not included in any statistical analysis.

FIGURE 10. TREATMENT COMBINATIONS USED IN RUBUS STRIGOSUS TEST

SEED SAMPLES	Warm Stratification ---------- Cold Stratification
	Scarification-- Warm Stratification-- Cold Stratification

Prior to the application of seed treatments, each seed sample was surface-sterilized with calcium hypochlorite 4% available chlorine) and then thoroughly washed with distilled water. All seed germination testing was done on sterile sand in pre-sterilized closed petri dishes. The germinator was maintained at a 20/30°C alternating temperature with light for eight hours during the high temperature period. The germination test duration was 35 days with germination counts made every seventh day. A seed considered as germinated in this experiment met the criteria for normal shrub seedlings of the Association of Official Seed Analysts (1978). All results are reported as real germination.

6.4 Results and Discussion

The total and cumulative germination results obtained in this experiment are presented in Figures 11 and 12 respectively.

The germination of unscarified red raspberry seed was significantly greater than in scarified treatments. This result and the difference in germination in unstratified samples between unscarified and scarified seeds (Table 3) indicate breaking the seed covers was not a beneficial pre-germination treatment. As reviewed

previously, Rose (1919) found scarificatioan had not been necessary to enable the seeds to imbibe water.

The experimental results do not allow any conclusions on the possibility of seed coverings causing dormancy through mechanical restriction to embryonic enlargement as hypothesized by Rose (1919) and subsequently cited by others in the literature (Adams 1927; Copeland 1976). However, when carrying out seed-cutting tests it was noted the endocarp and testa presented little resistance to cutting, even in unimbibed seeds.

Table 3

GERMINATION IN UNSTRATIFIED

RUBUS STRIGOSUS SAMPLES

Mechanical Scarification	Duration of Warm Stratification (Days)	Duration of Cold Stratification (Days)	Total Real Germination(%)
no	0	0	18
yes	0	0	1

This experiment and that of Scott and Ink (1957) both found warm stratification had a significant effect on germination. The results of these two studies indicated at least two-to-four months of warm stratification would be required to attain moderate levels of germination. Brinkman (1974) recommended a minimum of 90 days of warm stratification.

Germination was independent of the duration of the cold stratification treatments employed in the experiment. A comparison of the germination obtained in the unstratified samples and the experimental treatments implies cold stratification was generally of little benefit or detrimental in this seed lot. As previous research had also demonstrated red raspberry embryos were non-dormant (Rose 1919), it may be inferred cold stratification is not a necessary pre-germination treatment.

The germination testing regime used was that reported by Brinkman (1974). However, several modifications to the system may be considered. First, the data in Figure 12 and other information in the literature (Scott and Ink 1957) suggest a low rate of germination may be expected. Therefore, a longer test duration may be desirable. Secondly, the finding by Rose (1919) that the optimum germination temperature for red raspberry was 20-25^{o}C may indicate that the upper (alternating) temperature of 30^{o}C used here was excessive and that a lower maximum temperature may be preferable.

FIGURE 11. TOTAL GERMINATION IN *RUBUS STRIGOSUS* SEEDS

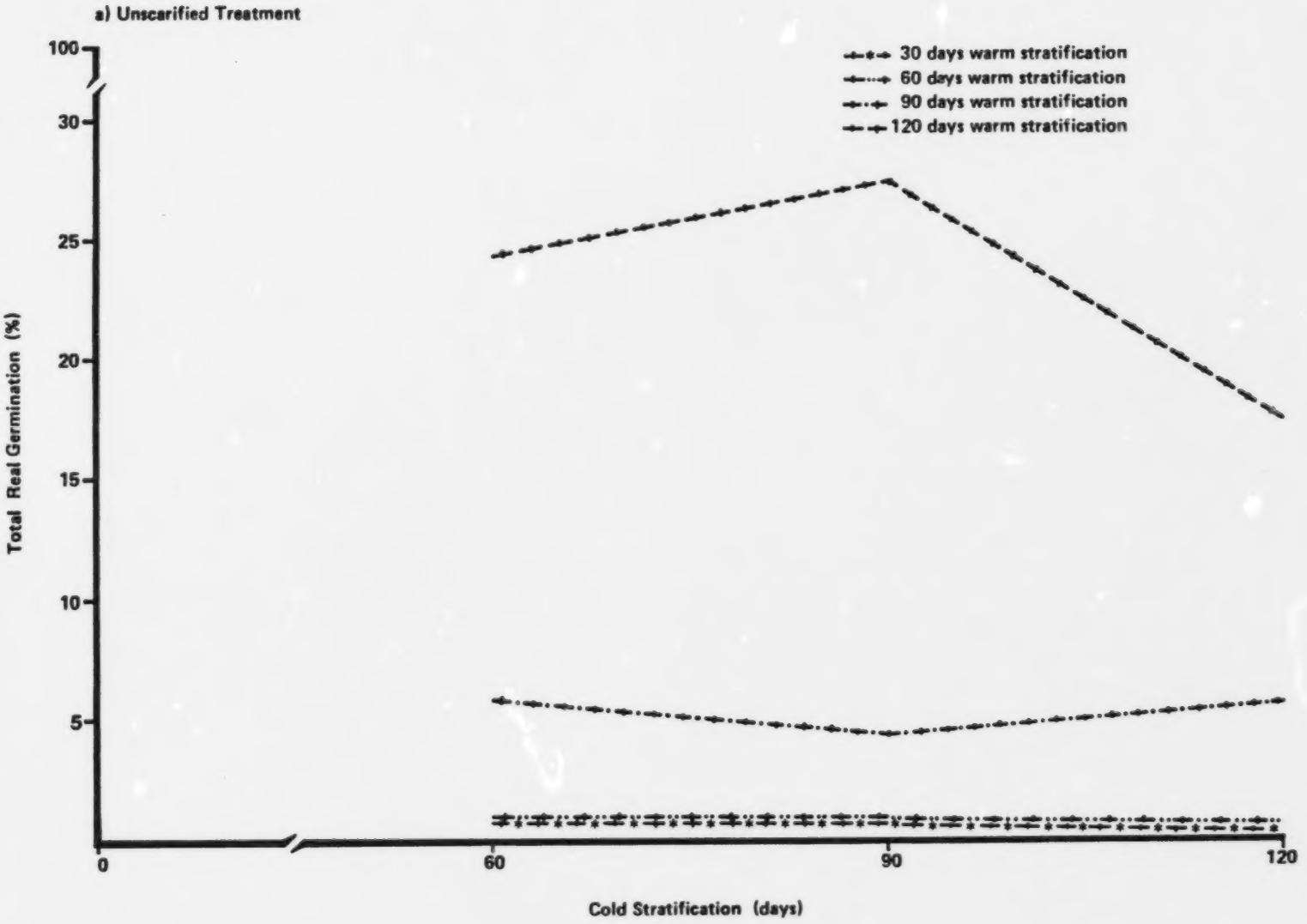


FIGURE 11. TOTAL GERMINATION IN *RUBUS STRIGOSUS* SEEDS

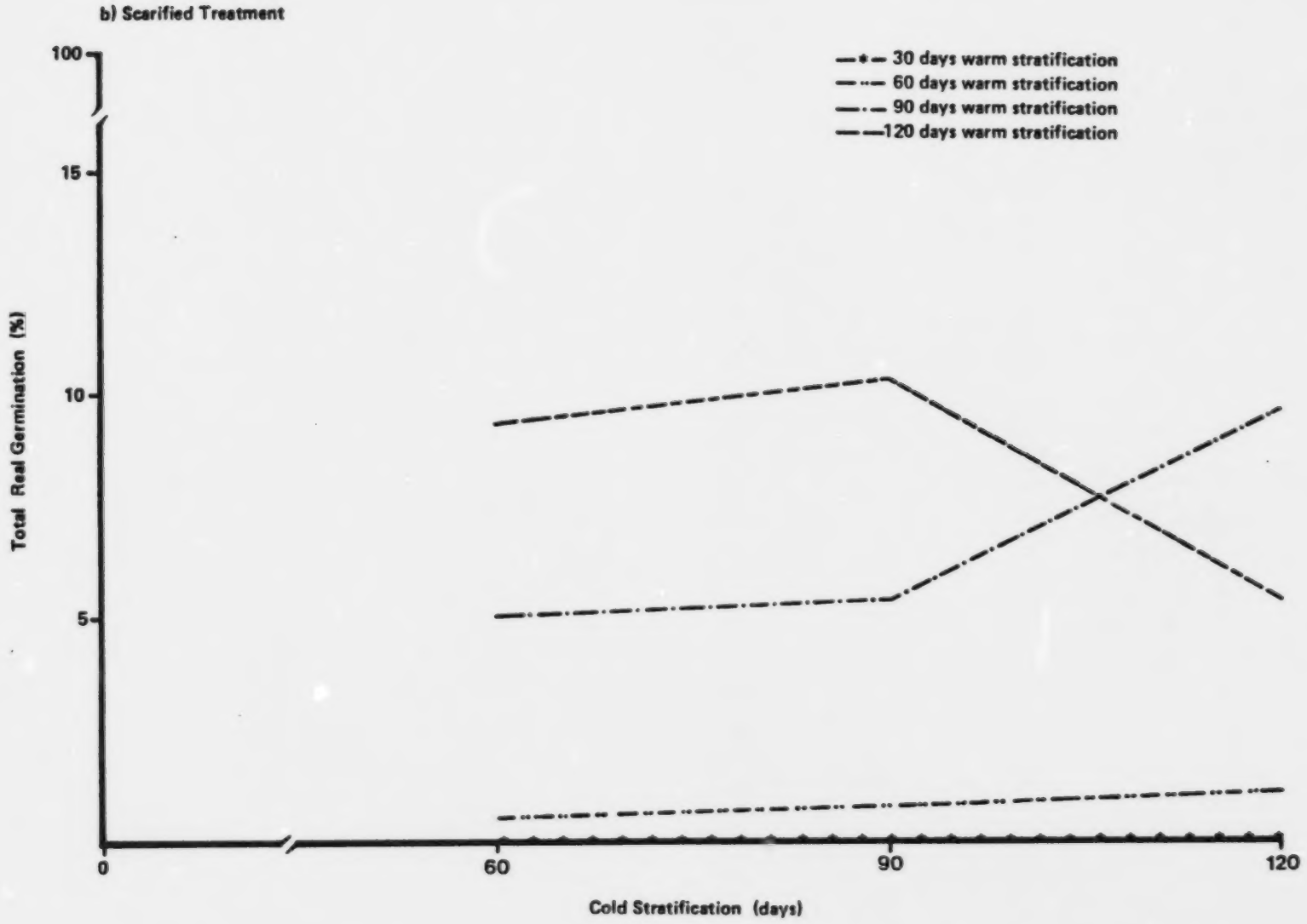


FIGURE 12. CUMULATIVE GERMINATION IN *RUBUS STRIGOSUS* SEEDS

a) 30 Days Warm Stratification

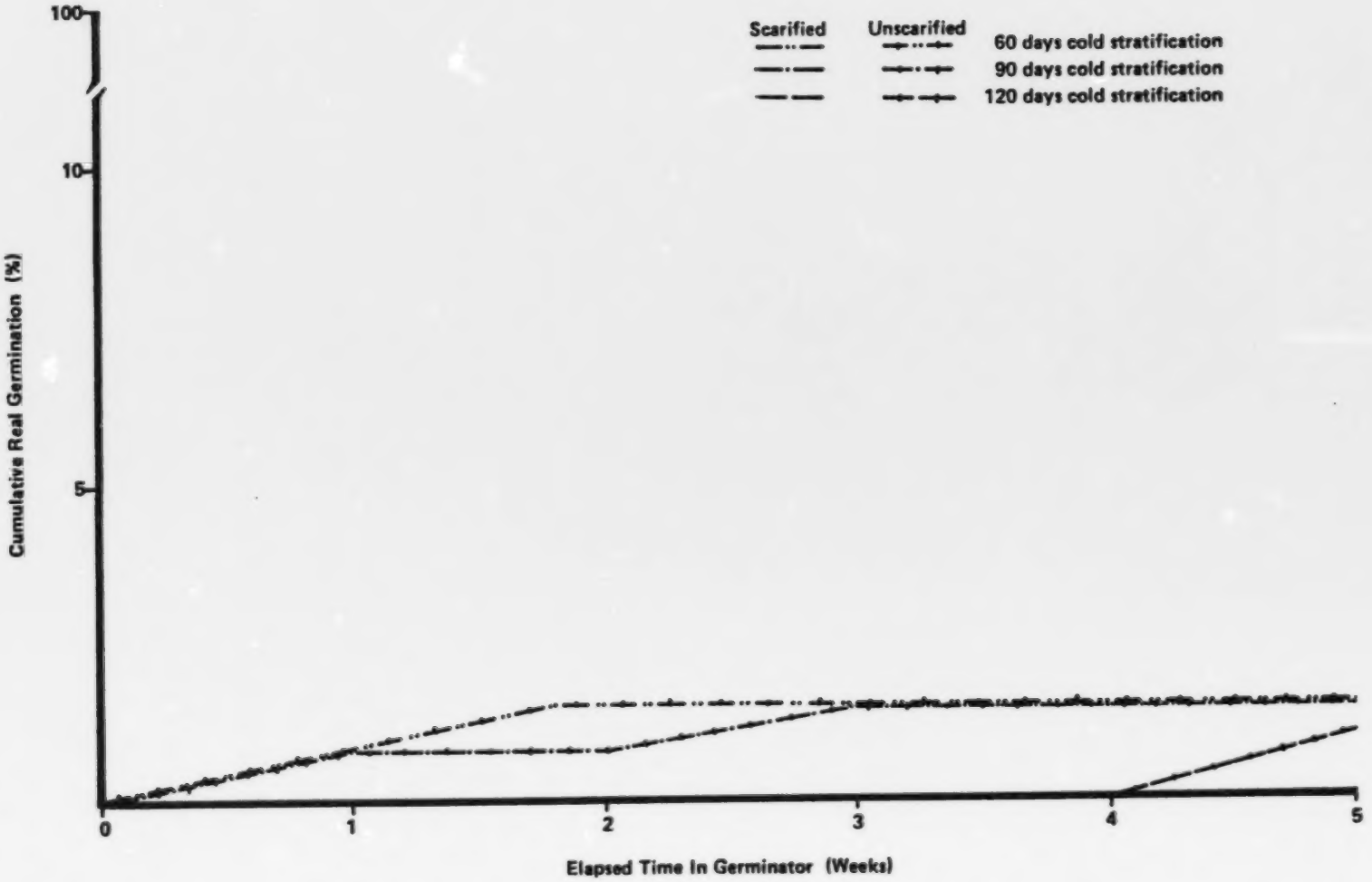


FIGURE 12. CUMULATIVE GERMINATION IN *RUBUS STRIGOSUS* SEEDS

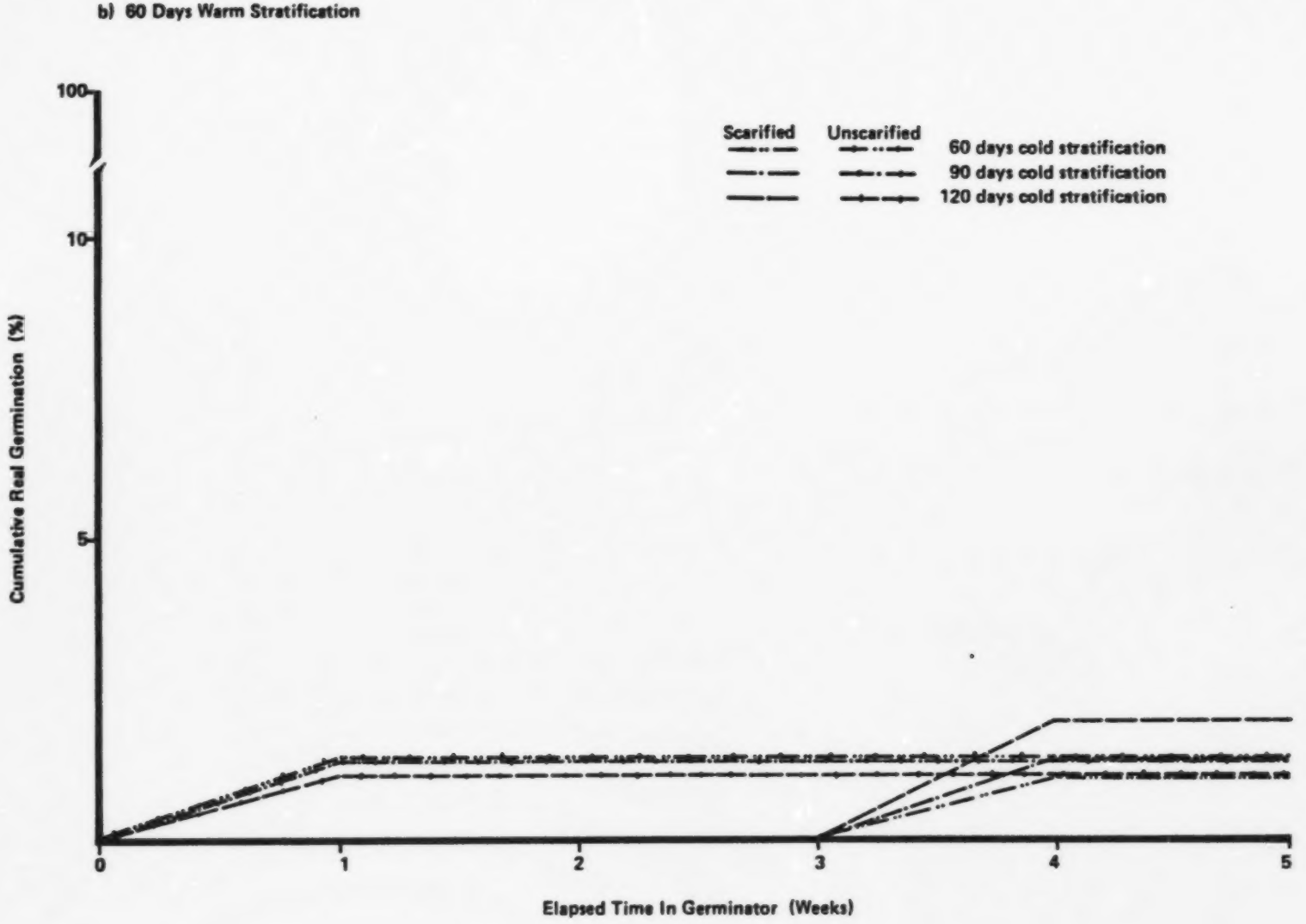


FIGURE 12. CUMULATIVE GERMINATION IN *RUBUS STRIGOSUS* SEEDS

c) 90 Days Warm Stratification

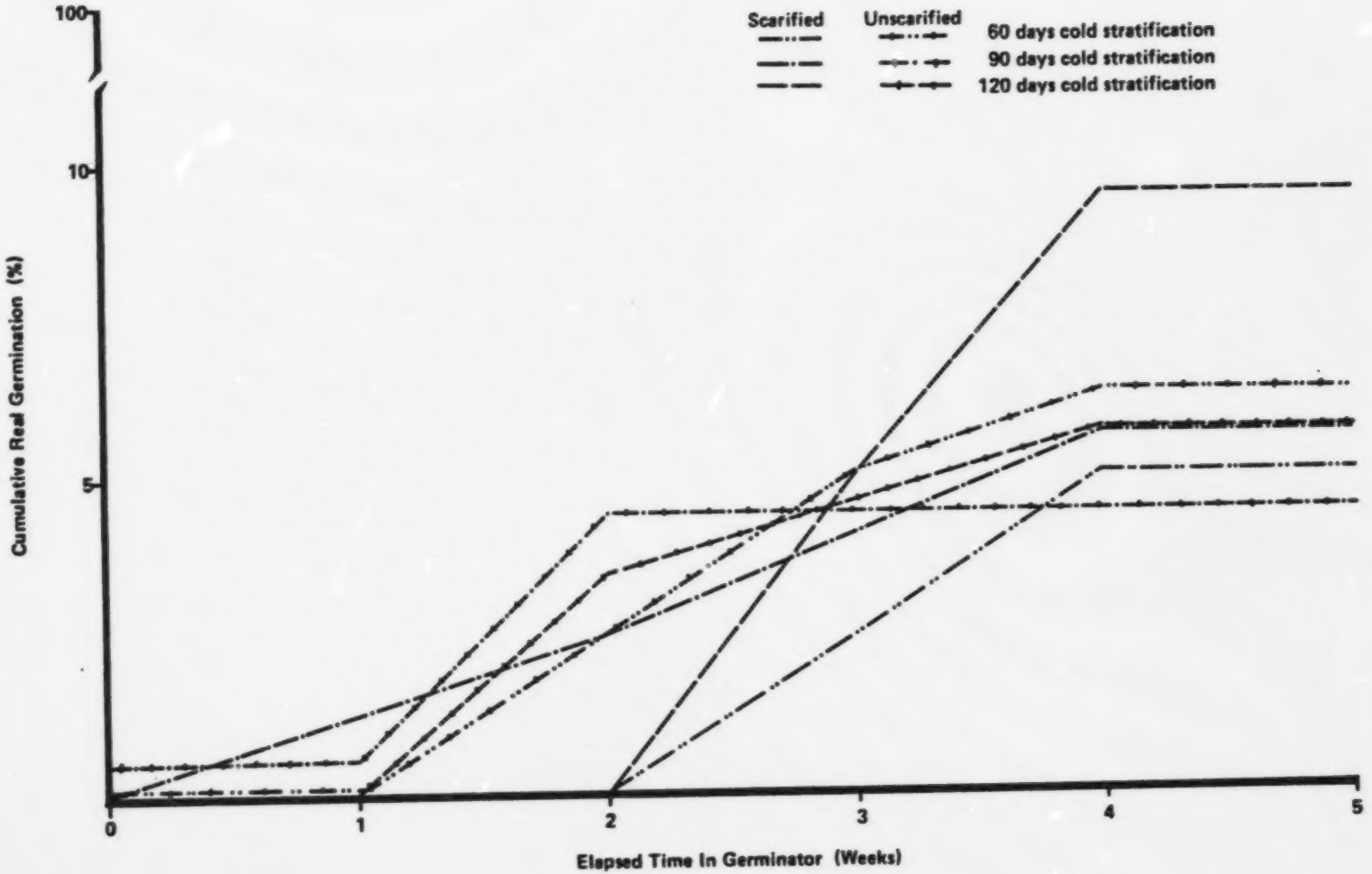
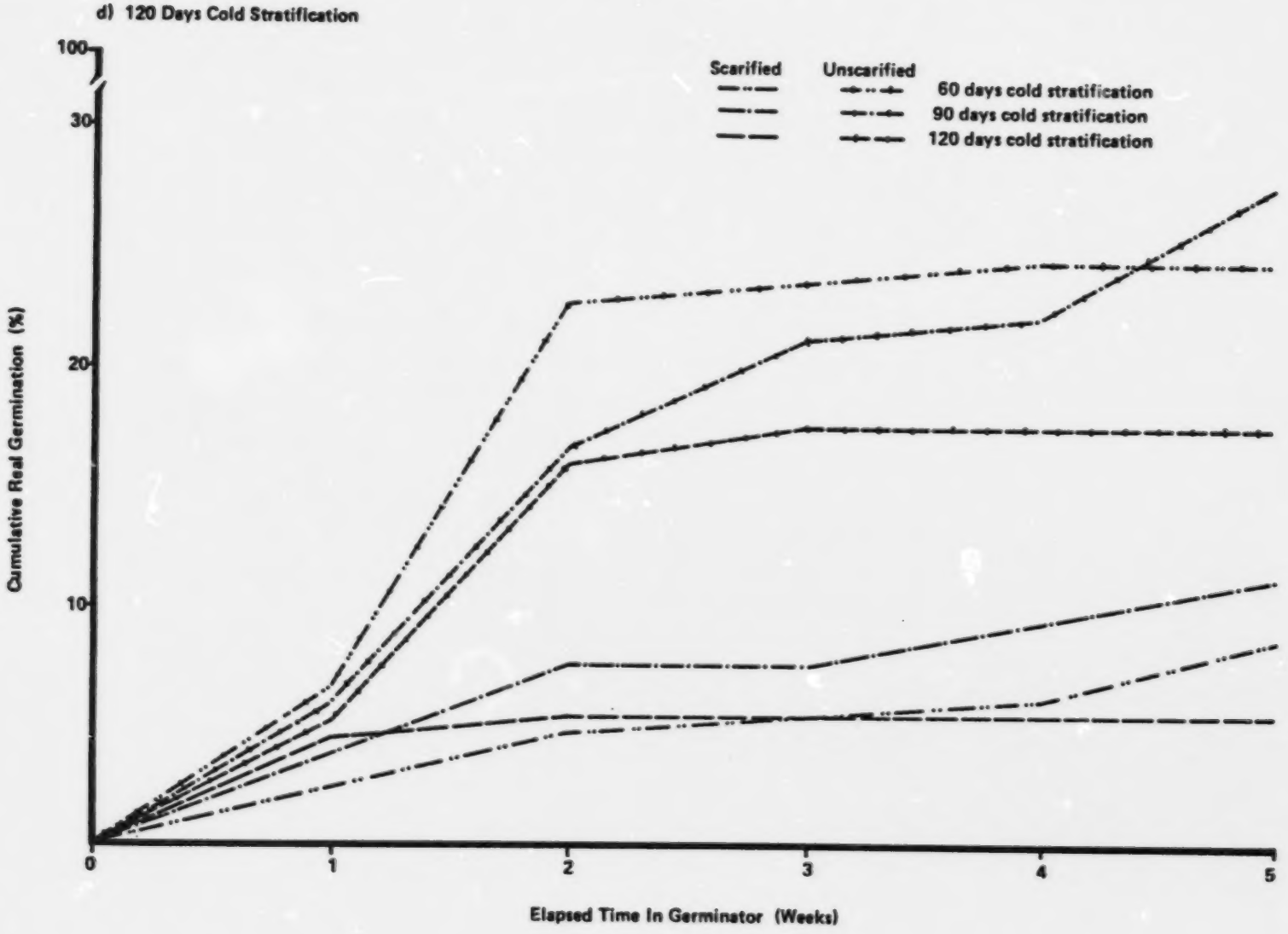


FIGURE 12. CUMULATIVE GERMINATION IN *RUBUS STRIGOSUS* SEEDS



7. ARCTOSTAPHYLOS UVA-URSI SEED PRE-GERMINATION TREATMENT TEST

7.1 Introduction

Arctostaphylos uva-ursi (L.) Spreng. (bearberry, kinnikinnik) is an evergreen prostrate shrub with branches of up to 15 cm in length (Vories 1981). It is common throughout the latitudinal range of the Eastern Slopes of Alberta, extending from the lower foothills zone into the alpine. Commonly found to have invaded disturbed sites (Russell 1979), bearberry is a species of fairly broad site adaptability (Sutton and Johnson 1974) and has potential for use in reclamation.

The fruit of A. uva-ursi is a mealy berrylike drupe containing four to 10 nutlets (Berg 1974; Vories 1981). The seed is composed of an embryo enclosed within a thick testa (Berg 1974).

7.2 Literature Review

The seed of A. uva-ursi is multiply dormant. Giersbach (1937) found entire seeds did not germinate while excised embryos germinated slowly and incompletely. The causes of dormancy are reported as hard seed and embryonic physiological immaturity (Berg 1974; Giersbach 1937; Glazebrook 1941; Heit 1967a; King 1947). Glazebrook (1941) attributed the seed coat dormancy both to impermeability to water and mechanical resistance to embryonic enlargement.

A number of early reviews recommended the use of cold stratification to break dormancy (Chadwick 1935; Shoemaker and Hargrave 1936). However, research carried out in Alberta has indicated this pre-germination treatment was ineffective (Simonson 1976; Fedkenheuer and Heacock 1980).

From the literature it would appear that dual or triple pre-germination treatments are required to obtain any level of germination. The dual treatment may consist of warm stratification followed by cold stratification (Babb 1959; Barton 1939; Vories 1981) or sulphuric acid scarification with a subsequent cold stratification (Babb 1959; Barton 1939; McKeever 1937). The triple treatment is a sequential sulphuric acid scarification - warm stratification - cold stratification (Berg 1974; Dick 1979; Giersbach 1937; Glazebrook 1941; King 1947; McLean 1967; Stark 1966). Some of these references were recommendations that did not present substantiating data. Those which were documented are summarized in Table 4. The results in the table indicate only low-to-moderate levels of germination may be expected in the species.

7.3 Methods and Materials

Mature fruit was collected October 10 to 12, 1982 at latitude 49°30'N, longitude 114°25' and an altitude of 1 670 m. After extraction and cleaning, the seed was stored in sealed containers at 5.8% moisture content and 0°C until treatments commenced.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The factors used in the design were:

a) scarification (seeds were non-scarified or mechanically scarified)
b) warm stratification for 0, 60, 90, and 120 days
c) cold stratification for 0, 60, 90, and 120 days.

Scarification was abrasive; sandpaper was used to remove a portion of the testa without damaging the embryo. The warm and cold stratification temperatures were 25°C and 5°C respectively. The stratification medium was sand.

Prior to the application of seed treatments, each seed sample was surface-sterilized with calcium hypochlorite (4% available chlorine) and then thoroughly washed with distilled water. All seed germination testing was done on sterile blotting paper in pre-sterilized closed petri dishes. The germinator was maintained

Table 4

SUMMARY OF REPORTED MULTIPLE PRE-GERMINATION TREATMENTS FOR

ARCTOSTAPHYLOS UVA-URSI

Reference	Maximum Germination Reported (%)	Time To Reach Maximum Germination	Treatments Which Resulted In Maximum Germination
Berg 1974	30-50	16 days	3-6 hours of sulphuric acid scarifica- ation followed by 60-120 days of warm stratification at 20-30°C followed by 60-90 days of cold stratification at 5°C
	61	16 days	6 hours of sulphuric acid scarification followed by 60 days of warm stratifi- cation at 25°C followed by 60 days of cold stratification at 5°C
Giersbach 1937	33	—	4 hours of sulphuric acid scarification followed by 4-5 months of warm strati- fication at 25°C followed by 10-15 months of cold stratification at 10°C
Glazebrook 1941	64	22 days	6 hours of sulphuric acid scarification followed by 60 days of warm stratification at 25°C followed by 60 days of cold stratification at 4°C
King 1947	10	15 days	8 hours of sulphuric acid scarification followed by 42 days of warm stratification at 20-24°C followed by 70 days of cold stratification at 1°C
McKeever 1938	6	7 days	3-5 hours of sulphuric acid scarification followed by 84 days of cold stratification at 5°C
McLean 1967	34	—	7 hours of sulphuric acid scarification followed by 90 days of warm stratification at room temperature followed by 90 days of cold stratification at 1°C.

at a constant 25°C temperature without light. The germination test duration was 21 days with germination counts made every seventh day. A seed considered as germinated in this experiment met the criteria for normal shrub seedlings of the Association of Official Seed Analysts (1978). All results are reported as real germination.

7.4 Results and Discussion

The total germination results obtained in the experiment are illustrated in Figure 13. In both unscarified and scarified treatments, seed which was not treated with warm and/or cold stratification did not germinate. No apparent consistent trend emerged in germination response to scarification, warm stratification and/or cold stratification. However, the peak germination (of 10%) obtained in the experiment was in scarified seed warm stratified for 120 days and then cold stratified for 90 days. The seed of this species has traditionally been difficult to propagate from seed (Berg 1974; Dick 1979; Shoemaker and Hargrave 1936).

A review of the procedures used in this experiment indicated several areas in which future work may be improved. First, as the type of stratification medium may have an effect on the biological activity during stratification and the medium used in this experiment was sand, it is suspected peat moss may be found to be more efficacious as a stratification medium. Secondly, the experiment did not employ any biological "quick test" for seed viability such as those specified by Grabe (1970) or the International Seed Testing Association (1966). The lack of high levels of germination obtained for A. uva-ursi in this and other reported experiments may be due to a large proportion of non-viable filled seeds. Thirdly, the germination test conditions employed in the experiment may not have been optimal; they were designed after those reported by Berg (1974) and McLean (1967). However, the information on germinator testing of bearberry is relatively deficient.

FIGURE 13. TOTAL GERMINATION IN *ARCTOSTAPHYLOS UVA-URSI* SEEDS

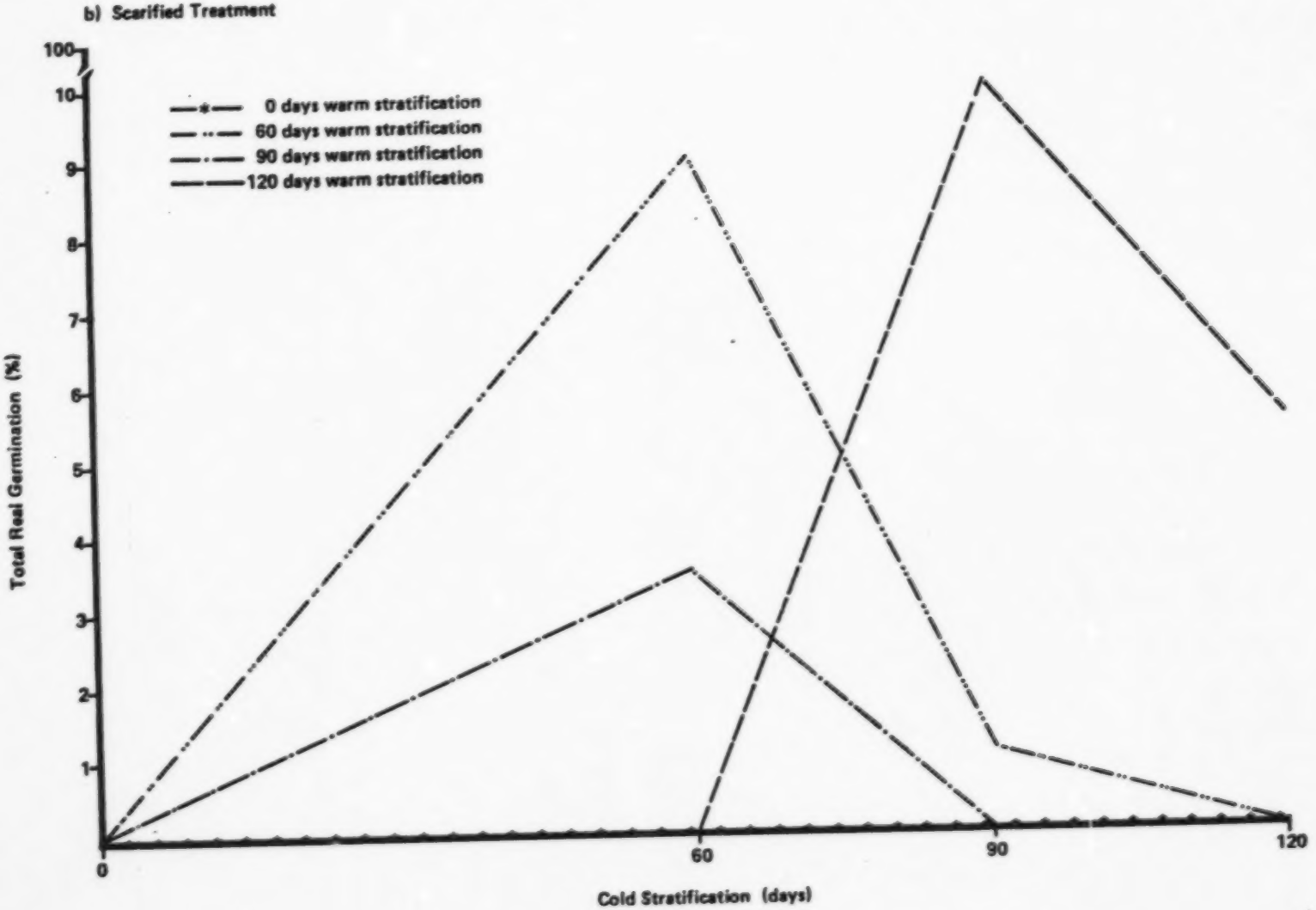
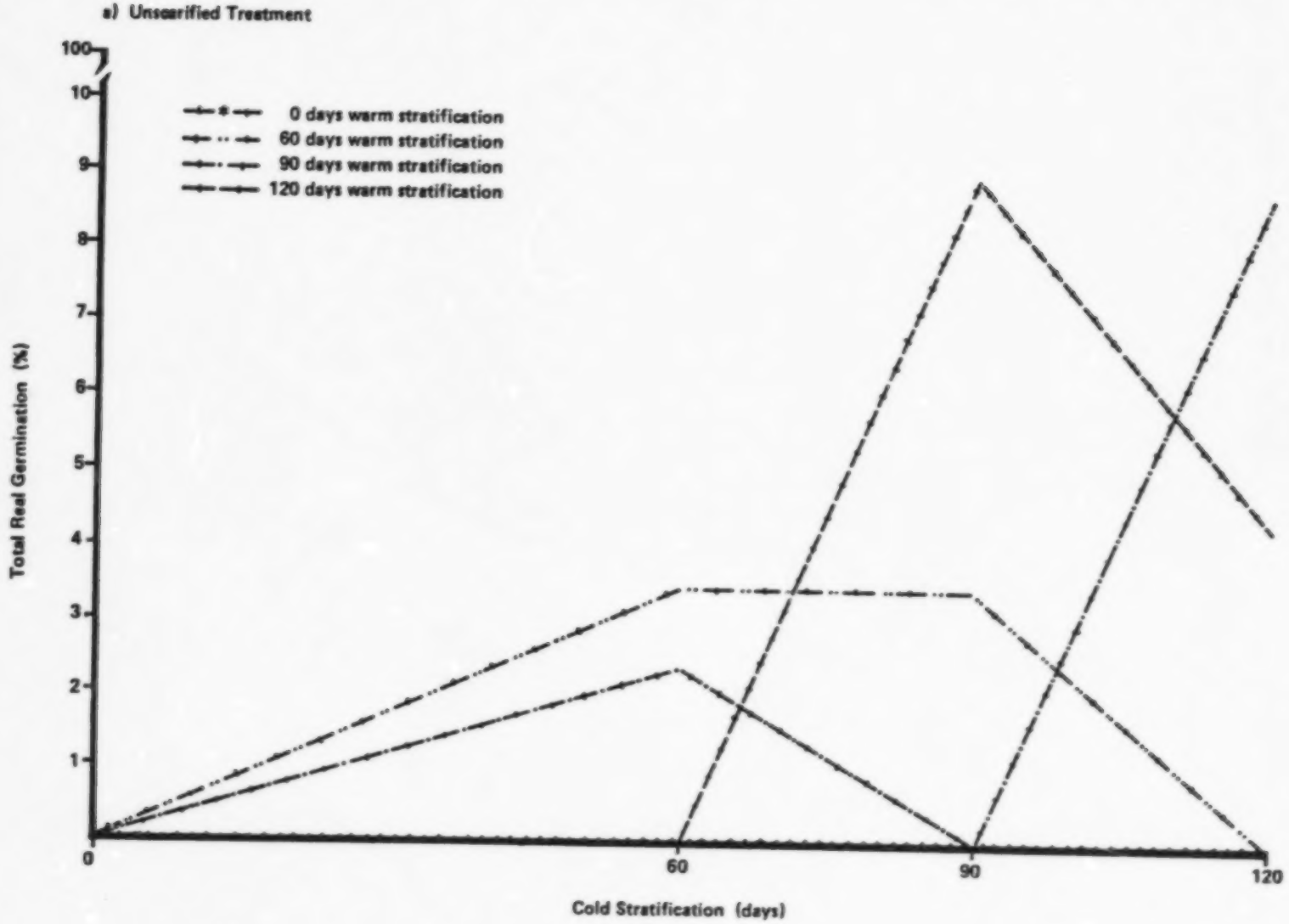


FIGURE 13. TOTAL GERMINATION IN *ARCTOSTAPHYLOS UVA-URSI* SEEDS



8. UNSUCCESSFUL SPECIES TESTS

In three of the selected species little or no germination was obtained in experiments. These are summarized in Table 5. The methodologies used in these three experiments are given in Appendix 2.

Table 5

MAXIMUM GERMINATION OBTAINED IN

UNSUCCESSFUL TESTS

Species	Maximum Total Germination Obtained (% Real)
Amelanchier alnifolia	2.6
Juniperus communis	0.0
Shepherdia canadensis	16.8

9. SUMMARY AND RECOMMENDATIONS

9.1 Elaeagnus commutata

Treatment of seeds with a cold running water leaching was the most efficacious pre-germination treatment with a 96-hour leach resulting in maximum total germination. The experimental results partially confirmed the conclusion of Corns and Schraa (1962): water-soluble germination inhibitors exerted the dominant influence on the germinability of silverberry seed.

Further research on water-leaching treatments is recommended. Such treatments should be composed of variations of leaching rates and durations. Consideration should also be given to inclusion of origin tests in the design.

Modifications must be made to the seed-testing regime. The test duration should be lengthened by at least several weeks. The germinator conditions of Olson (1974) may also prove to be superior.

9.2 Rosa acicularis

The experimental results demonstrated that prickly rose hip ripeness at the time of collection and extraction had an effect on seed germinability. They also suggested that the seed collections of the species may have been carried out earlier than has been traditional and before the hips had fully ripened. However, it should be noted that the stage of seed maturity is a critical consideration and, to date, the finding may have more biological than practical significance.

The germination of unstratified seed was not appreciable while the use of cold stratification alone yielded relatively low germination. Maximum levels of germination were obtained in

response to 60 days of warm stratification followed by 120 days of cold stratification.

Interpretation of the data and the discussion point to a number of areas for potential future investigation. These include further work on hip and fruit ripeness, methods of further enhancement of germinability, the physiological role of warm stratification, the effects of seed origin and annual variability in germinability. In the immediate future it is recommended that effort be concentrated on the first two.

In future seed work it is recommended that the duration of the germination test be shortened from the 12 weeks used in this experiment. Based on the experimental data, five weeks should be considered as the minimum length for such a test.

9.3 Rosa woodsii

The germination responses in Fendler woods rose were similar to those in R. acicularis. The maximum levels of germination across ripeness treatments were obtained in response to 60 days of warm stratification followed by 90 or 120 days of cold stratification. Both the duration of the dual pre-germination treatment and the level of germination obtained agreed with that reported elsewhere for the species (USDA 1979).

The recommendations are as given previously for R. acicularis.

9.4 Rubus parviflorus

In the experimental lot there was no benefit from scarification and it does not appear that impermeability was a cause of dormancy. Germination was independent of the cold-stratification treatments used in the trial, although it did appear that an undefined minimum duration of cold stratification was necessary in the species. Warm stratification for 30 to 60 days followed by cold stratification resulted in maximum germination. However, the physiological role of warm stratification was not known, although it may have been associated with chemical breakdown in the seed

coverings or initiation of metabolic processes requisite to germination.

Future work on the species may include the establishment of the minimum cold stratification requirement, the role of the endocarp and testa in seed dormancy, and the physiological role of warm stratification.

It does not appear that any major changes in the seed testing regime are necessary.

9.5 Rubus strigosus

Seed coat impermeability to water or gases did not appear to be a cause of dormancy in R. strigosus: in the experiment herein scarification treatment did not benefit germination while Rose (1919) reported that even entire seeds fully imbibed within a relatively short period. Cold stratification was generally of little benefit or detrimental to germination and may not have been a necessary pre-germination treatment. Warm stratification had a significant effect on germination and a minimum of two to four months of warm treatment was necessary.

Further research on the species should include examination of the role of the endocarp and testa in R. strigosus seed dormancy and might examine the physiological role of warm stratification in the species.

Some improvements in the testing regime are recommended. The test duration should be lengthened and the maximum germinator temperature used may be reduced to 20-25°C.

9.6 Arctostaphylos uva-ursi

In the past, low to moderate levels of germination have been obtained in this species. In this experiment no apparent trend emerged in response to the experimental treatments. However, maximum germination followed a combined treatment with scarification, warm stratification for 120 days and cold stratification for 90 days.

It is felt further work on the species would be worthwhile. Modifications in the scarification technique, stratification medium and testing regime are recommended. It is also suggested that viability testing be incorporated into any future work on the species.

9.7 Unsuccessful Species Tests

The seed pre-germination testing in *Amelanchier alnifolia*, *Juniperus communis* and *Shepherdia canadensis* were not successful.

It is recommended further work be carried out on *Amelanchier alnifolia* and *Shepherdia canadensis*. Seed propagation of *Juniperus communis* has traditionally been difficult and, at this point, it is doubtful that further investigation would be worthwhile.

APPENDIX 1

Analysis Of Variance For Elaeagnus commutata Total Real Germination

Source	df	SS	MS	F
Replicates	3	84.162	28.054	0.88
Leaching	3	2 968.367	989.456	30.97 * **
Stratification	4	3 383.376	845.844	26.47 * **
Leaching X Stratification	12	2 185.435	182.120	5.70 * **
Error	57	1 821.185	31.195	
TOTAL	79	10 442.525		

* significant at the 99% confidence level

** significant at the 95% confidence level

Analysis Of Variance For Rosa acicularis Total Real Germination

Source	df	SS	MS	F
Replicates	3	181.93	60.64	1.52
Warm Stratification	3	3 923.68	1 307.89	32.86 * **
Cold Stratification	3	17 186.28	5 728.76	143.72 * **
Ripeness	1	1 453.01	1 453.01	36.45 * **
Warm Stratification X Cold Stratification	9	3 206.30	356.26	8.94 * **
Warm Stratification X Ripeness	3	149.69	49.90	1.25
Cold Stratification X Ripeness	3	425.34	141.78	3.56 **
Warm Stratificaton X Cold Stratification X Ripeness	9	976.07	108.45	2.72 * **
Error	93	3 706.57	39.86	
TOTAL	127			

* significant at the 99% confidence level

** significant at the 95% confidence level

Analysis Of Variance For Rosa woodsii Total Real Germination

Source	df	SS	MS	F
Replicates	3	74.06	24.69	0.63
Warm Stratification	3	5 560.63	1 853.54	47.49 * **
Cold Stratification	3	16 384.93	5 461.64	140.22 * **
Ripeness	1	199.68	199.68	5.13 **
Warm Stratification X Cold Stratification	9	4 235.49	470.61	12.08 * **
Warm Stratification X Ripeness	3	151.15	50.38	1.29
Cold Stratification X Cold Stratification	3	119.70	39.90	1.02
Warm Stratificaton X Cold Stratification X Ripeness	9	396.08	44.01	1.13
Error	93	3 623.17	38.95	
TOTAL	127	30 744.89		

* significant at the 99% confidence level

** significant at the 95% confidence level

Analysis Of Variance For Rubus parviflorus Total Real Germination

Source	df	SS	MS	F
Replicates	3	252.91	84.30	1.76
Warm Stratification	3	764.50	254.83	5.40 * **
Cold Stratification	2	142.09	71.05	1.52
Scarification	1	6 439.96	6 439.96	136.44 * **
Warm Stratification X Cold Stratification	6	526.55	87.76	1.86
Warm Stratification X Scarification	3	203.24	67.75	1.44
Cold Stratification X Scarification	2	60.16	30.08	0.64
Warm Stratificaton X Cold Stratification X Scarification	6	760.47	126.69	2.68 **
Error	69	3 256.49	47.20	
TOTAL	95	12 406.37		

* significant at the 99% confidence level

** significant at the 95% confidence level

Analysis Of Variance For Rubus strigosus Total Real Germination

Source	df	SS	MS	F
Replicates	3	276.74	92.25	1.62
Warm Stratification	3	6 005.44	2 001.81	35.17 * **
Cold Stratification	2	288.55	144.28	2.54
Scarification	1	494.43	494.43	8.69 * **
Warm Stratification X Cold Stratification	6	37.24	6.21	1.09
Warm Stratification X Scarification	3	310.02	103.34	1.81
Cold Stratification X Scarification	2	222.28	111.14	1.95
Warm Stratificaton X Cold Stratification X Scarification	6	238.62	39.77	0.70
Error	69	3 927.24	56.92	
TOTAL	95	11 800.56		

* significant at the 99% confidence level

** significant at the 95% confidence level

Analysis Of Variance For Arctostaphylos uva-ursi Total Real Germination

Source	df	SS	MS	F
Replicates	3	79.12	26.37	2.71
Scarification	1	0.15	0.15	0.02
Warm Stratification	3	221.89	73.96	7.59 * **
Cold Stratification	3	160.18	53.39	5.48
Scarification X Warm Stratification	3	40.38	13.46	1.38
Scarification X Cold Stratification	3	44.01	14.67	1.51
Warm Stratification X Cold Stratification	9	648.70	72.08	7.39
Scarification X Warm Stratification X Cold Stratification	9	155.04	17.03	1.75
Error	93	906.60	9.75	
TOTAL	127	2 256.07		

* significant at the 99% confidence level

** significant at the 95% confidence level

APPENDIX 2

Methodology For Amelanchier alnifolia Test

Saskatoon fruit was collected August 18 and 19, 1980 at latitude 49°20'N, longitude 114°20' and an altitude of 1 555 m. Two ripeness phases were gathered. In the first, referred to as "not fully ripe", the fruit was uniformly reddish-purple in color. The second, designated as "fully ripe", had dark purple fruits. In both the seeds were in the hard dough stage. After extraction and cleaning the seed lots were dried to 5.8 - 6.0% moisture content and then stored in sealed containers at 0°C.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The factors used in the experiment were:

a) ripeness (as described previously)
b) cold stratification for 0, 60, 75, and 90 days.

The cold stratification temperature was 4°C. The stratification medium was horticultural peat with a pH of 5.8.

Prior to the application of stratification treatments, each seed sample was surface-sterilized with a calcium hypochlorite solution (4% available chlorine), then thoroughly rinsed with distilled water. All seed-germination testing was done on sterile moist blotting paper in pre-sterilized closed petri dishes in a germinator. The germinator was maintained at a constant 21°C without light. The germination test duration was 28 days with germination counts made every seven days. A seed considered as germinated in this experiment met the criteria for normal seedlings of the Association of Official Seed Analysts (1978).

Methodology For Juniperus communis Test

Juniper fruit was collected November 14, 1980 at latitude 53°30'N, longitude 117°35' and an altitude of 1 060 m. After extraction and cleaning, the seed was stored in sealed containers at 6.1% moisture content and 0°C until testing commenced.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The factors used in the experiment were:

a) warm stratification for 0, 45, 60, 75, 90, and 105 days
b) cold stratification for 0, 60, 90, 120, and 150 days.

The warm and cold stratification temperatures were 25°C and 5°C respectively. When dual stratification treatments were used, warm stratification was preceded by cold stratification. Horticultural peat moss with a pH of 5.8 was used as the stratification medium.

Prior to the application of stratification treatments, each seed sample was treated with a 50% lye solution and surface-sterilized with a calcium hypochlorite solution (4% available chlorine) and then thoroughly rinsed with distilled water. All seed germination testing was done in a germinator on sterile blotting paper in pre-sterilized closed petri dishes. The germinator was maintained at 20°C with light for 16 hours per day. The germination test duration was 28 days with germination counts made every seven days. A seed considered as germinated in this experiment met the criteria for normal shrub seedlings of the Association of Official Seed Analysts (1978).

Methodology For Shepherdia canadensis Test

Mature buffalo berry fruit was collected July 25 and 26, 1980 at latitude 52°10'N, longitude 116°25' and an altitude of 1 325 m. After extraction and cleaning, the seed was stored in sealed containers at 6.1% moisture content and 0°C until testing commenced.

The experiment was a factorial design employing four replicates per treatment. Each replicate consisted of a randomly selected 50-seed sample. The main effects used in the design were:

a) scarification (seeds were non-scarified or mechanically scarified)
b) warm stratification for 0, 10, 20, and 30 days
c) cold stratification for 0, 15, 30, 45, and 60 days.

The warm and cold stratification temperatures were 20°C and 5°C respectively. When dual stratification treatments were used, warm stratification preceded cold stratification. The stratification medium was horticultural peat moss with a pH of 5.8.

Prior to the application of stratification treatments, each seed sample was surface-sterilized with a calcium hypochlorite solution (4% available chlorine) and then thoroughly rinsed with distilled water. All seed germination testing was done on sterile moist blotting paper in pre-sterilized closed petri dishes placed in a germinator. The germinator was maintained at a 20°/30° alternating temperature with light during the high temperature period. The germination test duration was 21 days with germination counts made every seventh day. A seed considered as germinated in this experiment met the criteria for normal shrub seedlings of the Association of Official Seed Analysts (1978).

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